International Symposium

The Hypophyseal Growth Hormone, Nature and Actions

The Symposium was sponsored by the Henry Ford Hospital and Edsel B Ford Institute for Medical Research Detroit, Michigan and held at the Hospital October 27 28 29 1954

Program Committee

Edwin E Hays	Paul D Bartlett
C N H Long	Oliver H Gaebler
Choh Hao Li	C Paul Hodgkinson
Ephraim Shorr	Joseph A Johnston
Alfred E Wilhelmi	Richmond W Smith Jr

International Symposium

The Hypophyseal Growth Hormone, Nature and Actions

Ed tors

RICHMOND W SMITH JR M D

Physician in Charge Division of Endocrinology
Henry Ford Hospital Detroit

OLIVER H GAEBLER M D

Head Biochemistry Department
Edsel B Ford Institute for Medical Research Detroit

C N H LONG M D

Sterling Professor of Physiology
Yale University School of Medicine New Haven

The Blakiston Division

McGRAW HILL BOOK COMPANY INC

New Yo k Toro to Lo don

THE HYPOPHYSEAL GROWTH HORMONE
NATURE AND ACTIONS

Copyright © 1955 by the McGraw Hill Book Company Inc
Printed in the United States of America All rights reserved
This book or parts thereof may not be reproduced
in any form without permission of the publishers

Library of Congress Catalog Card Number 55 8643

Participants

Astwood Edwin B	*Boston Massachusetts*
Baker Burton L	*Ann Arbor Michigan*
Bartlett Paul D	*Detroit Michigan*
Bennett Leslie L	*Berkeley California*
Best Charles H	*Toronto Canada*
Campbell James	*Toronto Canada*
Cole Roger D	*Berkeley California*
Copp D Harold	*Vancouver Canada*
Cori Carl F	*St Louis Missouri*
de Bodo Richard C	*New York New York*
Dougherty Thomas F	*Salt Lake City Utah*
Ellis Stanley	*Berkeley California*
Elrick Harold	*Denver Colorado*
Engel Frank L	*Durham North Carolina*
Fell Honor B	*Cambridge England*
Folley S J	*Shinfield England*
Gaebler Oliver H	*Detroit Michigan*
Geschwind Irving I	*Berkeley California*
Greenbaum A L	*London England*
Haist Reginald E	*Toronto Canada*
Hisaw Frederick L	*Cambridge Massachusetts*
Houssay Bernardo A	*Buenos Aires Argentina*
Kinsell Laurance W	*Oakland California*
Krahl Maurice E	*Chicago Illinois*
Lee Milton O	*Washington D C*
Li Choh Hao	*Berkeley California*
Long C N H	*New Haven Connecticut*
Lukens F D W	*Philadelphia Pennsylvania*
McHenry E W	*Toronto Canada*
Moore Francis	*Boston Massachusetts*
Park Charles R	*Nashville Tennessee*
Raben Maurice S	*Boston Massachusetts*
Randle Philip J	*Cambridge England*
Reid Eric	*London England*
Riddle Oscar	*Plant City Florida*
Russell Jane A	*Emory University Georgia*
Segaloff Albert	*New Orleans Louisiana*
Selye Hans	*Montreal Canada*
Shaw J C	*College Park Maryland*
Shorr Ephraim	*New York New York*
Simpson Miriam E	*Berkeley California*
Washburn Alfred H	*Denver Colorado*
Weiss Paul	*Washington D C*
White Harvey L	*St Louis Missouri*
Wilhelmi Alfred	*Emory University Georgia*

Introduction

In this volume are recorded the complete proceedings of an international symposium *The Hypophyseal Growth Hormone Nature and Actions* The symposium was held at the Henry Ford Hospital on October 27 28 and 29 1954 and was sponsored jointly by the Hospital and the Edsel B Ford Institute for Medical Research with their respective staffs It was the second in a series of such meetings inaugurated by Dr Robin Buerki Executive Director as an impetus to progress in the basic medical sciences The first of these was held in 1953 and its proceedings were published by The Blakiston Company Inc in 1954 under the title of *The Dynamics of Virus and Rickettsial Infections* For the 1955 meeting the subject *Enzymes Units of Biological Structure and Function* has been selected by the joint staffs of the Institute and Hospital

The symposium on the growth hormone was attended by 300 persons from laboratories and institutions in Europe England South America Canada and throughout the United States Among them were representatives of many scientific disciplines including anatomists physiologists biochemists pharmacologists zoologists and investigators in endocrinology animal husbandry and clinical medicine not to name them all This broad representation had much to do with the unquestioned success of the meeting

Regardless of its ultimate value the symposium was in certain respects a notable occasion This was apparent to those who realized that the enigmatic pituitary growth hormone had been under study by one full generation of investigators and that present at the symposium were all the scientists with few exceptions who had been intimately concerned at one time or other with this most elusive of the pituitary hormones It was 33 years ago that J A Long and H M Evans detected growth promoting activity in crude extracts of the anterior hypophysis Shortly thereafter the series of experiments by P E Smith began defining the various functions of the anterior pituitary and the pursuit of the growth promoting factor had begun Closely following these developments was the first of the many important observations of Houssay on the relation of anterior pituitary action to diabetes This is not the place to review the history of anterior lobe physiology nor is it adequate to highlight just certain milestones along the 30 year road of scientific effort However for those who were familiar with the historical background it was a memorable experience to have witnessed the joint participation of such eminent investigators As is apparent from the list of participants on page v of this volume two scientists were conspicuously absent The reader undoubtedly will have identified them as Herbert M

Evans and F G Young Dr Evans was to have addressed the symposium following the dinner on the first evening and to have reminisced on the past 30 years or more of investigation in the field of the pituitary hormones A temporary illness forced him from participating Dr Young was fulfilling long standing commitments in South Africa at the time of the meeting but his laboratory and philosophies were ably represented by his associate, P J Randle At this point it is appropriate to mention the contribution to the symposium by Alfred H Washburn who addressed the symposium members and many of the hospital staff following the dinner on the second night He summarized certain of the impressive number of observations on patterns of human growth that he has accumulated over the past 20 years

A few comments about the programming and symposium operations are warranted inasmuch as these are reflected closely in the format of the present volume As will be noted the symposium was divided into 5 Parts and between some of these there is an apparent overlapping of headings as for example in the subject designations of Part II and IV Each is concerned with the growth hormone effects on certain structures yet it should be apparent that effects on morphology is for the most part the essential topic of Part II while the mechanism of action is the main theme of Part IV Another reason for the apparent arbitrariness of subject grouping was the desire of the Program Committee to devote one full day to the extensive material on the major metabolic actions of growth hormone (Part III) This meant some division of other areas of study which might have been grouped under one heading It will be apparent to the reader that the large amount of research concerning the action of growth hormone on the mammary gland warranted assigning this material to a part which would permit an uninterrupted consideration Inasmuch as the data on growth hormone effects in man were quite limited and since much of the work both in this area and in respect to the mammary gland was applied or clinical research it was decided to combine these into Part V

This volume was prepared for publication with the view that the recorded data methods and speculations pertaining to this accelerating field of research should be passed on without delay to the many readers anticipating its release In order to accomplish this it was decided that all contributions to the symposium having been carefully recorded would be revised by one editor and that the material would not be submitted to the respective speaker or discussor for his own revisions Obviously this introduced the considerable hazard of misinterpreting the thought or data which the contributor actually intended to present particularly in the designated and general discussion periods This is mentioned in order to free the participants of any responsibility for the misinterpretations which may have resulted Likewise proofreading was the sole responsibility of the editors and the authors should be relieved of criticism for any errors in the publication of the manuscripts

Considerable importance has been attached to the discussion periods which followed each group of papers With few exceptions a designated speaker initiated the discussion period either presenting data and observations from his own experience or discussing the previous presentations All invited guests were encouraged to contribute in the general discussion period and all comments were fully recorded It is hoped that the reader will devote equal attention to the portions of the volume devoted to the general discussions Only certain brief remarks of the chairmen which concerned the mechanics of running the symposium have been deleted

Finally it might be said that the symposium on the growth hormone did not arise from circumstances which have led to many of the valuable conferences we have witnessed in the past few years Growth homone to date, has shown discouragingly little clinical promise and the symposium was primarily a meeting of investigators working in the basic biological and medical sciences Possibly the status of the pituitary growth hormone is about where corticotropin quietly rested in 1946 1947 and 1948 prior to the notable observations of Hench Kendall and others which opened the floodgates to applied research and clinical trials with corticotropin Although as this conference revealed there is little at the present time to encourage the idea that growth hormone will be of similar usefulness in clinical medicine nevertheless this possibility is one that cannot be ignored For the moment it will be rewarding enough to convince ourselves that there is a specific pituitary growth hormone that it can be isolated intact and in amounts which will permit extensive experimentation and that it does manifest anabolic properties in man Perhaps our thinking has become conditioned to the rapid metabolic actions of corticotropin and we expect unreasonably that growth hormone will induce immediate and readily measured changes If we consider the growth process we will appreciate that even under optimal circumstances it proceeds at limited rates Broadly characterized normal growth is a subliminal steady and usually proportionate phenomenon The limits of our yardsticks must be heeded and it would be artless science to expect in short term experiments quantitative, or even qualitative duplications of naturally occurring growth This symposium was held to sift over the rapidly accumulating data to compare techniques of study to attempt a resolution of disturbing differences and to stimulate new approaches which someday may bring usefulness to this most intriguing pituitary substance

We wish to gratefully acknowledge the splendid secretarial assistance rendered by Miss Dorothy Reid and Mrs Nancy Hasegawa and the enthusiastic support given to the Program Committee by the administrative and service departments of the hospital

RICHMOND W SMITH JR
OLIVER H GAEBLER
C N H LONG

Detroit Michigan
February 1955

Contents

II

Effects of Growth Hormone on Certain Structures

Chairman

Frederick L Hisaw

III

Growth Hormone and Energy Sources

Chairman

F D W Lukens

IV

Growth Hormone and Cellular Systems

Chairman

Carl F Cori

V

Influence of Growth Hormone on the Mammary Gland and on Human Metabolism

Chairman

Oscar Riddle

Part I

Bioassay, Preparation and Physicochemical Properties of Growth Hormone

Chairman

C N H Long
Yale University School of Medicine
New Haven Connecticut

1

What is Growth?*

Paul Weiss
Rockefeller Institute for Medical Research New York

The invitation to give an introductory address to this meeting is a distinct honor it also carries a mandate to set the phenomenon of growth in proper perspective before discussing growth hormone Unfortunately growth itself has received much less critical attention than have the agents for which it serves as indicator and assay To put it bluntly growth is a term as vague ambiguous and fuzzy as everyday language has ever produced Adopted into scientific language without precise and consistent meaning it may be passable for crude description but is ill fitted to analytical application Hence if you ask Just what is Growth? the correct answer is A word that covers like a blanket a multitude of various things and meanings To know growth for what it really is rather than what we are wont to call it we must remove that blanket and uncover the underlying facts it has concealed This I propose to do in rudimentary sample form as time permits A close look at the facts will do far more for clarification than would a host of academic definitions and circumlocutions

Our notions about growth have been shaped more by usage than by incisive study they form a sort of scientific folklore As a result we find that various groups while they all just plainly speak of growth do not all mean and talk about the same thing Thus growth has come to connote any and all of these reproduction increase in dimensions linear increase gain in weight gain in organic mass cell multiplication mitosis cell migration protein synthesis and perhaps more It would seem inconsistent to apply the most exacting standards of precision to our research data and then proceed to mix into their description and interpretation such vague ter

* Research supported by grants in aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council and from the National Institutes of Health Public Health Service

minology as this The mixture can be no more precise than its vaguest ingredient [1]

Then what is wrong? Why such diversity of views and versions? The reasons lie in our unfounded expectation that growth is a single, simple measurable entity In this conviction each of us has tended to deal with his own limited aspect of the problem as if it were a representative sample of the total perspective Yet far from being a single simple and unitary phenomenon growth is conglomerate, complex and intricate and this is why it defies formulation in simple terms What usually deceives us is the sim plicity of our tools and terms of measurement which all too easily produce the illusion of similar simplicity of the measured systems

Just bear in mind how we get to know about growth by taking measure ments at different times comparing them and noting a net gain—of size or mass or numbers These serial measurements then define a growth curve as descriptive of the particular system as let us say a fingerprint—and equally empirical This is the blanket under which a host of disparate events lie hidden events moreover of opposite signs some adding to others sub tracting from the measured body Since they are not all of one kind and their shares are unequal growth can be recorded but it cannot be under stood without identifying these tributaries and determining their respective contributions

Let me phrase this in terms of an analogy The body is a community of cells each cell a community of smaller particles and each particle an assemblage of molecular species Thus the proper analogue of biological growth is the growth of a human community for example of a city Here we rate as growth for instance any increase in population over a given interval But a simple tally will not tell us how the increase has come about It takes census data to give a more detailed accounting They reveal that additions come from two different sources reproduction from within and immigra tion from without losses likewise from death as well as emigration The results would be altogether different if instead of just counting noses we chose to include in our considerations the physical wealth of the community that is the net gain in goods and estates produced by the members of the population To understand its sources would require running inventories of raw materials, production conversion consumption imports exports stor age and wastage Moreover in either reckoning the data can have meaning only in reference to fixed boundaries which divide what we count as within from that which we count as without

Now as we apply this simile to biological growth the whole indefinite ness of our customary position becomes obvious First let us consider the matter on the tissue level Suppose we note an increase in the number of cells of a given organ What does this really tell us? As in the human popu lation some cells have reproduced others have immigrated still others have been lost by shedding or disintegration the proportions and rates of these

component events varying from tissue to tissue The final tally—no more than a crude balance sheet—discloses none of these details According to Hamburger and Levi Montalcini for instance, abnormal enlargements in the early central nervous system formerly ascribed simply to hyperplasia that is overproduction are partly due to the fact that fewer cells degenerate rather than that more are being proliferated and partly to the fact that the cell group being counted has received additions from an indifferent pool outside the counted area Another shortcoming of plain cell counts is that they ignore all growth of individual cells (e g hypertrophy) not followed by division

If then we turn from cell counts to over all dimensions or total mass we are on even weaker ground In terms of our community analogy we first have to agree as to what is and what is not real property of the system we measure or what has been acquired and what discarded during the measured period Food in the alimentary tract is still distinctly out of bounds even if stored for weeks as in a hamster s pouch But what of this mass once it has passed into the blood and lymph stream? Though strictly on the inside it still has not become converted into substance of the body proper Then what about the food stuffs stored in modified form for instance as glycogen or fat in liver or fat bodies? Their fluctuations up and down are not conventionally considered growth and degrowth Why? Because we sense that growth connotes some *permanent* addition and merely temporary physiological variations do not qualify under this title

Then what about the wastes not yet eliminated? And the products manufactured by our organs? Take hair or nails or even red cells—terminal products destined to be shed or otherwise eliminated In counting bodily productions is it fair to include just those fractions which happen to be present on the measured body when we take our measurements and leave out all the unknown mass that has been similarly produced in the interim but irretrievably lost? Evidently we ought to be consistent and either count it all in or all out neither of which is practicable We certainly would not collect secretions such as slime urine sweat and sebum over a measured period and add them to the growth record Yet we do customarily include the bulk of cartilage and bone and other connective tissues which consists of residues of cellular secretions just like those other ones but incidentally deposited instead of extruded hence accruing to the measured mass Thus what we measure is related not so much to the process of production as to the accident of the disposal of the products If they persist we count them if they drop out we miss them

The arbitrariness attached to our measurements is about the same whether we use total mass dry weight nitrogen content volume length or what not as reference system It is even worse when we turn from the body to its component cells The cell is bounded by a surface and we are in the habit of ascribing any increase in the volume thus enclosed to growth But

minology as this The mixture can be no more precise than its vaguest ingredient [1]

Then what is wrong? Why such diversity of views and versions? The reasons lie in our unfounded expectation that growth is a single simple measurable entity In this conviction each of us has tended to deal with his own limited aspect of the problem as if it were a representative sample of the total perspective Yet far from being a single simple and unitary phenomenon growth is conglomerate complex and intricate and this is why it defies formulation in simple terms What usually deceives us is the simplicity of our tools and terms of measurement which all too easily produce the illusion of similar simplicity of the measured systems

Just bear in mind how we get to know about growth by taking measurements at different times comparing them and noting a net gain—of size or mass or numbers These serial measurements then define a growth curve as descriptive of the particular system as let us say a fingerprint—and equally empirical This is the blanket under which a host of disparate events lie hidden events moreover of opposite signs some adding to others subtracting from the measured body Since they are not all of one kind and their shares are unequal growth can be recorded but it cannot be understood without identifying these tributaries and determining their respective contributions

Let me phrase this in terms of an analogy The body is a community of cells each cell a community of smaller particles and each particle an assemblage of molecular species Thus the proper analogue of biological growth is the growth of a human community for example, of a city Here we rate as growth for instance any increase in population over a given interval But a simple tally will not tell us how the increase has come about It takes census data to give a more detailed accounting They reveal that additions come from two different sources reproduction from within and immigration from without losses likewise from death as well as emigration The results would be altogether different if instead of just counting noses we chose to include in our considerations the physical wealth of the community that is the net gain in goods and estates produced by the members of the population To understand its sources would require running inventories of raw materials production conversion consumption imports exports storage and wastage Moreover in either reckoning the data can have meaning only in reference to fixed boundaries which divide what we count as within from that which we count as without

Now as we apply this simile to biological growth the whole indefiniteness of our customary position becomes obvious First let us consider the matter on the tissue level Suppose we note an increase in the number of cells of a given organ What does this really tell us? As in the human population, some cells have reproduced others have immigrated still others have been lost by shedding or disintegration the proportions and rates of these

component events varying from tissue to tissue The final tally—no more than a crude balance sheet—discloses none of these details According to Hamburger and Levi Montalcini[2] for instance abnormal enlargements in the early central nervous system, formerly ascribed simply to hyperplasia that is overproduction are partly due to the fact that fewer cells degenerate rather than that more are being proliferated and partly to the fact that the cell group being counted has received additions from an indifferent pool outside the counted area Another shortcoming of plain cell counts is that they ignore all growth of individual cells (e g hypertrophy) not followed by division

If then we turn from cell counts to over all dimensions or total mass we are on even weaker ground In terms of our community analogy we first have to agree as to what is and what is not real property of the system we measure or what has been acquired and what discarded during the measured period Food in the alimentary tract is still distinctly out-of bounds even if stored for weeks as in a hamster s pouch But what of this mass once it has passed into the blood and lymph stream? Though strictly on the inside it still has not become converted into substance of the body proper Then what about the food stuffs stored in modified form for instance as glycogen or fat in liver or fat bodies? Their fluctuations up and down are not conventionally considered growth and degrowth Why? Because we sense that growth connotes some *permanent* addition and merely temporary physiological variations do not qualify under this title

Then what about the wastes not yet eliminated? And the products manufactured by our organs? Take hair or nails or even red cells—terminal products destined to be shed or otherwise eliminated In counting bodily productions is it fair to include just those fractions which happen to be present on the measured body when we take our measurements and leave out all the unknown mass that has been similarly produced in the interim but irretrievably lost? Evidently we ought to be consistent and either count it all in or all out neither of which is practicable We certainly would not collect secretions such as slime urine sweat and sebum over a measured period and add them to the growth record Yet we do customarily include the bulk of cartilage and bone and other connective tissues which consists of residues of cellular secretions just like those other ones but incidentally deposited instead of extruded hence accruing to the measured mass Thus what we measure is related not so much to the process of production as to the accident of the disposal of the products If they persist we count them if they drop out we miss them

The arbitrariness attached to our measurements is about the same whether we use total mass dry weight nitrogen content volume length or what not as reference system It is even worse when we turn from the body to its component cells The cell is bounded by a surface and we are in the habit of ascribing any increase in the volume thus enclosed to growth But

water, electrolytes food stuffs and wastes pass in and out across that boundary without revealing to the observer just when they lose their original small molecular identity to become merged with the complex specific compounds of the cell or when they emerge again from decomposition of the latter we thus cannot determine what fraction of a given increase to allocate to protoplasmic synthesis proper and what fraction to substances that just reside within the confines of the cell but strictly speaking belong to it either not yet or no longer comparable to food and wastes in the alimentary tract Then how should we consider the formed cell inclusions which vary according to each type of cell? Obviously the production of myofibrils which accumulate within the muscle cell thus adding to its mass is not fundamentally different from the production of collagen fibrils which leave their cell of origin—the fibroblast—hence do not enlarge it Conversely a fat cell inflated by unextruded fat of its own production cannot be compared to a macrophage similarly dilated by fat from stuff it has engorged from the outside Evidently the attainment of equal or unequal sizes as such can give us little information about the identity or difference of the underlying causes of the enlargement

It is this habit of confining ourselves deliberately to a single parameter of a highly complex system—for instance mass—and then measuring all changes on this single scale only which tempts us as I said before to confound the arbitrary simplicity of our method with an inherent simplicity of the object itself We are fundamentally in error if we prorate the average over all changes in a complex system evenly over all parts of the system as if all of them took equal shares in the result In doing so we commit an act of mental homogenization as it were obliterating the organized complexity of the heterogenous events which are the very center of our interest

To this we usually add a second and similarly unfounded simplification whenever we consider growth rates We measure two comparable parameters—let us say weight or length—at the beginning and end of a convenient period divide the difference by the time elapsed and call the quotient growth rate Empirically this gives useful descriptive data But before using these for analytical or comparative purposes we should realize that we have again homogenized in this case the time interval We have tacitly assumed that during this interval the bracketed change has been steady and continuous Yet if the change occurred unevenly in spurts separated by phases of quiescence any inference as to the kinetics of the system would be misleading Two different systems can achieve the same amount of growth increment in precisely the same period by wholly different means the one by growing faster for shorter spells the other growing more slowly but with fewer interruptions or shorter lags

But this example also contains the clue to overcoming our predicament You note that the two systems just mentioned would become readily distinguishable if they were sampled at shorter intervals that is by letting

factual information replace supposition and extrapolation And this is a lesson which cannot be overstressed Interpretations and comparisons in matters of growth will remain of little value and validity if they are based on over all generalizations instead of such detailed and painstaking sorting and recording of the component events as is practically feasible Tally must give way to detailed census There are insuperable limitations set to this census by our ignorance of cell life and the inadequate resolving power of our tools but it would be unpardonable not to carry the analysis at least as far as the objects and techniques would permit

Therefore turning to practical correctives let me indicate how a first breakdown of the problem could be attempted Growth is the surplus accruing in the balance sheet of a complex account Our task is to itemize the accounting Here are some major items In listing them I shall artificially separate them as if they were consecutive steps while in reality you understand they proceed more or less concurrently (Fig 1)

We follow a system through a period of growth from A to G The system may be a cell an organ an individual Now in the first place we must distinguish within each such system between two major fractions—a reproductive and a non reproductive one the former capable of giving rise to more of its own kind—more protoplasm in a cell or more cells in a tissue—the latter merely dead bulk in point of growth—fibers granules and all sorts of functional equipment in a cell or fully differentiated non proliferating

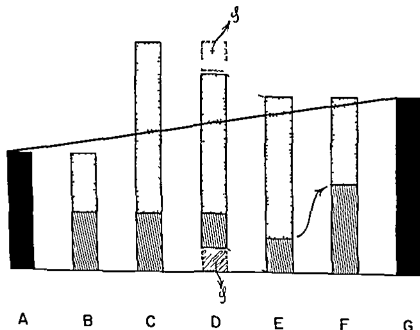


Fig 1

cells in a tissue Their proportions vary according to cell type, species, age and activity In our example, about 50% are assumed to be reproductive as shown (stippled) in B During the recorded growth period this mass will increase to the dimensions shown in C At the same time, however there will occur some losses from degradation lysis, shedding extrusion emigration and so forth These will reduce both fractions by the amounts indicated in D The resulting state E has larger mass than the initial A You note however that the proportion between the reproductive and non reproductive fraction comes out markedly altered In most cases this is then rectified by the conversion of freshly reproduced protoplasm into sterile differentiated products as indicated in F As you can readily see this conversion which drains the reproductive potential of the system is of crucial influence on what we externally discern as growth rate In the end we find our system in the enlarged state G The point I want to stress with this diagram is that even assuming the simplest case namely proportionate growth the result cannot be interpreted to mean that all parts have taken part in growth let alone grown at equal rates or taken equal shares in the composite effect And nothing short of detailed study will tell the real story

This confronts us with a major task which we mostly dodge rather than face dodge by attaching real biological meaning to such purely descriptive terms as growth stimulation or depression rather than face by finding out just *why* a given system, subject to certain agents turns out to be either

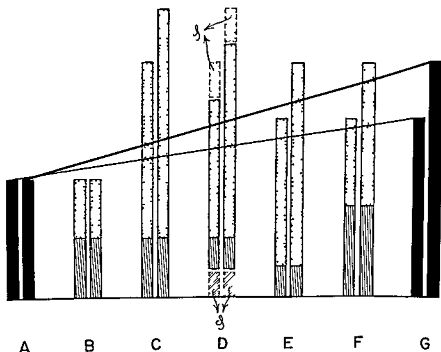


Fig 2

larger or smaller than a chosen reference standard For this is all we usually determine and yet we imply far more Just let us take for once a closer look Let us return to the diagram of Figure 1 and assume that it represents a normal reference condition Suppose we now doctor an identical system with an agent let us say growth hormone that entails an even greater increase during the observed period (Figs 2 3 4) We sum up our observation by stating that growth has been stimulated like an invested principle that suddenly yields higher returns because the interest rate has gone up Yet greater gain may come not only from faster earning through stepped up production but also from reduced consumption or even from diverting less of the working capital to non productive uses These various possible modes of action of our agent are illustrated in the remaining diagrams in which the left one of each pair of bars always gives the control data from Figure 1 while the right one represents the experimental case

In Figure 2 we actually let the velocity or intensity of reproduction be increased (from B to C) This alone naturally leads to faster growth but at the same time it also modifies the distribution of reproductive and sterile mass (compare the two bars in F) which if uncorrected would distort the whole growth pattern So even this seemingly simple change is not so simple as it seems

The second alternative is shown in Figure 3 Here we assume that our so-called stimulating agent has simply reactivated part of the normally quiescent fraction to become reproductive (in B) thus recruiting a larger

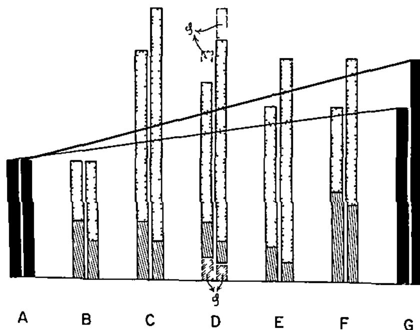


FIG 3

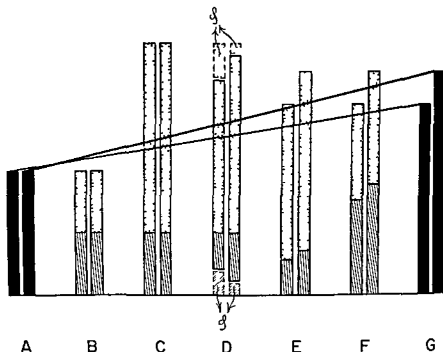


Fig 4

source for active growth without however stepping up the growth rate of the elements at all The target process here would be the conversion of reproductive mass to specialized products Reduce or retard this conversion and you have the picture of growth stimulation

Lastly the same picture would be obtained (Fig 4) if merely the debit side of the balance sheet of growth is reduced (in D) that is when there is less than the normal amount of destruction consumption and dissipation

There are many further variants of this complex account but these few here will have served our purpose to show that the same net increase can be brought about by a variety of unrelated means hence that comparing different systems or different agents on the sheer basis of net increases observed may lead or rather mislead to quite gratuitous interpretations Perhaps in such critical illumination the term growth hormone may eventually turn out to have been overly suggestive

But to leave this critical and somewhat defeatist note what is there in the way of positive information that could give sharper focus to the growth problem? However fragmentary this information still is it begins to piece together The tentative result may be summarized in the following seven theses

(1) *The general common denominator shared by all organic growth is protoplasmic reproduction* which involves the replication of those high molecular systems in each cell that are characteristic of the particular cell

type and are compounded only inside of cells of that kind It is important however, to make a clear distinction between primary protoplasmic repro duction and secondary elaboration of protoplasmic products All such cell products including the fibers and ground substances whose deposits form the bulk of most bodies are derived from protoplasm secondarily either by direct transformation or by synthesis with the aid of enzymes which in turn have originated in the process of protoplasm synthesis The current shorthand habit of equating protoplasmic reproduction with protein syn thesis apart from by passing the non proteinaceous constituents of proto-plasm fails to take this fundamental distinction into account For instance, while such proteins as collagen or melanin cannot themselves give rise to more collagen or melanin under any known conditions the protoplasms of the fibroblast or melanoblast in their growth evidently multiply the chemical machineries that can synthesize more collagen or melanin Thus only a fraction of the cellular protein is actually engaged in protoplasmic reproduc tion the rest is sterile but just what those fractions are remains to be deter mined In the following I shall use the term growth in the restricted sense of protoplasmic reproduction

(2) *Growth in the indicated sense has its sources not uniformly dis tributed throughout the cell but is of localized origin centering on the nuclear territory* The nuclear source of protoplasmic reproduction has been deduced from cytochemical studies[3] and demonstrated by our own experi mental studies on neurons[4] The nucleated part of the nerve cell body issues continuously a fresh supply of neuroplasm which wicklike moves periph erad in the nerve fiber to replenish basic protoplasmic systems at the rate at which they are breaking down by ordinary wear and tear Evidently although any reasonably large fragment of a nerve fiber can carry out com plex metabolic functions and syntheses by virtue of its enzyme content the enzyme systems themselves cannot be reproduced ubiquitously but must be furnished from the nuclear supply center Although even the mature neuron maintains itself in a state of perpetual growth renewal not all somatic cells can be assumed to retain this faculty Those that have lost it are destined to die precociously and they can be replaced only from those reserves that still have it In the epidermis for instance the squamous outer cells are in the former state the basal cells in the latter A single neuron could be viewed as comparable to a vertical column of epidermal cells (the peri karyon corresponding to the basal cell) in which cell boundaries had become obliterated

(3) *The nuclear growth process is intimately related to perhaps en gendered by the multiplication of chromosomal genes* As a result cell growth is closely associated with nuclear growth Accordingly haploid nuclei and cells with only half the normal complement of chromosomes are of half the normal size whereas polyploid nuclei and cells with multiple chromosome sets acquire correspondingly excessive sizes Parenthetically

there is by no means a binding relation between cell size on the one hand and organ and body size on the other while haploid organisms are dwarfs because they are made of dwarf cells in normal number polyploid organs and animals often remain of normal dimensions as the giant size of their cells is offset by the use of smaller numbers[5] Although true cell growth is reflected in nuclear increase the reverse is not always true, and nuclear enlargement as such is not sufficient evidence of true growth but may be simply a sign of functional hyperactivity The two types of increase can sometimes be told apart cytochemically as true growth is accompanied by augmentation of nuclear DNA whereas hyperactivity is not[6] DNA seems to assume ever increasing significance as index of true growth[7]

(4) *Despite the fact that the genic equipment according to current thought is essentially the same in all somatic cells mode and rate of growth of each cell type are specific for that type* that is, neuroplasm begets more neuroplasm myoplasm more myoplasm thyroplasm more thyroplasm and so forth To deal with this matter which touches on the complex problem of differentiation is beyond our scope here We mention it merely because it reveals that genic replication while seemingly the initiating step in the growth process is followed by chains of events the nature of which is deter mined by the specific constitution of the (extragenic) rest of the cell and in this regard the different cell types differ crucially Besides certain basic prerequisites in common to all of them each type has its private needs for instance in tissue culture, one tissue will grow better in one kind of medium while another tissue thrives better in another kind usually without our knowing just why Understandably the more conspicuous common factors necessary for all growth have received more attention Those basic to the maintenance of life in all cells whether growing or non growing have no particular relevance to the growth problem as such But up and above these common prerequisites for life are the special needs of growing versus non growing systems and the differential needs of different growing systems according to their kinds These include specific building blocks factors to insure the proper physical framework for growth and perhaps separate energy resources and enzyme systems to support growth in contradistinc tion to stationary maintenance Signs are increasing that the growth process is not a mere quantitative shift of the normal steady state between anabolism and katabolism in favor of the former but a process of different character sustained by auxiliary biochemical systems dormant during states of sheer maintenance At least this is a conclusion suggested by metabolic studies on forms in which periods of maintenance and growth are clearly separated as in some insects[8]

In view of the qualitative and quantitative differences between the growth requirements of different tissues and organs we must concede to each one its characteristic chemical kinetics And this explains the following familiar experience

(5) *Given optimal conditions superabundance of all prerequisites and freedom from active inhibitions each cell strain tissue and organ grows at its own characteristic rate* This rate represents the maximum output of which the particular system is capable (at a given temperature) It is a ceiling rate and varies according to species genetic constitution kind of tissue and organ state of differentiation and perhaps age It manifests itself *for instance in the fact that* transplants between different species or breeds of different growth rates if successful at all continue to grow according to their native growth patterns[9]

Now under normal conditions in the body tissues do not grow at ceiling rates This shows that conditions are not usually optimal for total output either because of bottlenecks in the supply of some essential elements which may be a matter of timing or of competition for limited supplies or because of suboptimal physical conditions or lastly because of the presence of factors that actively repress or retard one step or another in the reaction chain Now as you note any of these situations by holding growth to a level below its potential ceiling gives the external appearance of an inhibitory influence and is likely to be so labelled Yet between them they have no more in common than the various agents that may cause an automobile to stall Reversing the argument any lessening of the effectiveness of any one of them may enable the system to come closer to its optimal growth output and if so will give us the impression of stimulation a more correct term would be desinhibition Some recent work of ours strongly supports this view[10] In the epidermis of starved amphibians cell growth followed by mitosis can be evoked in two different ways (a) throughout the skin by sudden feeding or (b) in a localized spot by fragments of certain organs inserted under the skin The magnitude of the unit response is the same for (a) or (b) alone and for (a) and (b) combined This demonstrates the existence of a ceiling On the other hand when the same agents are applied at any stage at which growth is submaximal a new peak of growth activity appears which again has the height of the ceiling This evidently proves that these agents have acted not by boosting the growth process by a given amount as would be the connotation of stimulation but by releasing the system from certain depressive effects that had previously been in operation

Obviously factors restraining growth by holding any of its contributory steps to a suboptimal level belong to a great variety of categories and need have nothing in common except their eventual effect on the growth result Similarly then the agents which offset these restraining factors thereby reversing the net effect on growth are likewise manifold and varied One might thus question whether the search for over all growth stimulators is at all realistic and promising

To judge from virus reproduction the multiplication of basic protoplasmic units presumably takes up no more than a very minor fraction of the time

needed for a given growth interval, let us say, the interphase between two somatic mitoses where according to the DNA index the crucial events seem to be crowded toward the end [11] The rest of the time is evidently used for processes preparing this crucial step as well as for the subsequent elaboration of differentiated products Since these vary from one cell type to another any observable shortening of interphase—appearing as growth stimulation —is due more probably to the speeding up of some of the prefatory or consecutive steps rather than of the crucial act of genic reproduction

(6) *Growth and cell division are often but not necessarily coupled* Present evidence indicates that in this correlation cell growth is the primary event with mitosis then supervening facultatively In such cases and with due caution mitosis may be used as index of preceding growth Even then comparisons are difficult for in all cases in which only a fraction of the cell population takes part in proliferation a rise of the mitotic index need not signify either a shortening of interphase or a protraction of the mitotic act but simply the mitotic involvement of a larger portion of the population Again speaking of 'growth stimulation would add little to our insight

(7) *As complex as the growth process itself are the means that keep the various steps of the process, as well as the various growth centers among one another in mutual harmony* It cannot be stressed too strongly that growth is regulated by a great multiplicity of factors of chemical and structural nature no single one of master rank [1] One potent growth regulating principle which we discovered relatively recently is a feed back equilibration between a growing organ on the one hand and organ specific discharges of its own production that restrain the growth of all cells of the homologous type on the other Each cell type may be assumed to give off substances specific to its kind which inhibit the multiplication of any protoplasm of the same type in proportion to their concentration in the extracellular environment As this concentration increases with the growing mass of an organ growth of each cell type will become self limiting regardless of how widely dispersed the total mass is throughout the body This principle explains the compensatory growth reactions after partial removal or injury of a given organ as well as the observation that the presence of crushed cells of a given type in the blood stream (by injection) or in a culture medium enhances the growth of homologous cells for a more detailed review of the evidence [1] While this principle operates among homologous members of a cell and organ population growth effects between heterogenous types are a major function of hormones

* * *

Since growth processes are so diverse and composite it would be a miracle if any one hormone were to act on all of them in such fashion as

to net always a positive balance which would make it a veritable growth hormone Any hormone affecting any one of the innumerable component steps of growth is in a sense a growth hormone although nothing seems to be gained by naming it so The obligation remains to find out precisely where and how it acts This requires more than just measuring over all changes of size or bulk or composition

To illustrate the danger of shorthand explanations of the relation of hormones to growth let me conclude with an instructive example from our own laboratory experience Amphibian metamorphosis from larva to adult is as you know dependent upon thyroid secretion The hormone activates a pattern of proliferation and involution processes and all organs undergo profound reorganization including the brain In this process a peculiar pair of giant hindbrain cells concerned with larval swimming so called Mauthner s cells atrophy while other brain parts grow This could have been ascribed to the loss of their functional terminations but our experiments[13] proved otherwise We implanted fragments of thyroid or thyroxin soaked agar above the 4th ventricle so as to allow the hormone to diffuse into the brain wall according to earlier results [14] under such conditions a circumscribed metamorphosis of the tissue complex within the diffusion field could be expected This actually occurred with the following results on brain growth while all other ganglion cells of the area grew conspicuously that one pair of cells in their midst which was destined to regress—Mauthner s cells—did not grow but shrank (Fig 5) We could show that this reverse behavior had nothing to do with peculiarities of size or position but was simply an expression of a different biochemical constitution of these cells which predisposed them to react to thyroid hormone with a growth response of opposite sign from that of the other cells of the group In conventional terminology one would say that the same hormone in the same dosage at the same time can induce either hypertrophy or atrophy depending on the kind of cell it strikes If this holds true for cells so closely related as the different neurons how much more generally will it apply to more widely

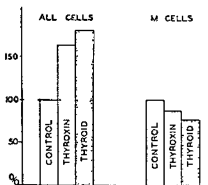


Fig 5

diversified cell populations It seems hardly necessary to labor the cautionary lesson contained in this example

*To sum up what we measure as growth is the resultant of a heterogeneous array of processes of most diverse kinds and there is no way of telling in advance without careful analysis just what component may be affected by any particular agent in any particular tissue in any particular species and how As long as we keep this in mind we are on safe ground even if for convenience we resort to such shorthand reference terms as the one heading this conference Even if the growth hormone should turn out not to be just what its name implies I doubt whether this would in the least detract from the superb factual achievements in its study of which we are to hear an up to date account just as I doubt that progress in X rays would have been delayed if they had instead been named death rays because undeniably they sometimes kill I also have little doubt that those with firsthand experience in this field are essentially aware of what the real situation is and how complex it is Yet there are also those whom the simple label growth might delude into holding complacent oversimplifi and unrealistic notions of its content My comments were intended to restore the complex problem to plain view—not for discouragement but simply for clarification of the problems so that research may orient itself toward the real thing instead of to a verbal symbol As for my final answer to What is Growth? I am tempted to dodge by saying Let us go back to work and find out more about it and not pretend we know

References

1 Weiss P *Chemistry and Physiology of Growth* Ed A K Parpart Princeton University Press 1949 135
2 Hamburger V and R Levi Montalcini *Genetic Neurology* Ed P Weiss University of Chicago Press 1950 128
3 Caspersson T O *Cell Growth and Cell Function* New York W W Norton & Co Inc 1950 185
4 Weiss P and H B Hiscoe *J Exp Zool* **107** 315 (1948)
5 Fankhauser G *Quart Rev Biol* **20** 20 (1945)
6 Schrader F and C Leuchtenberger *Exp Cell Research* **1** 421 (1950)
7 Swift H H *International Review of Cytology* Eds Bourne G H and J F Danielli New York Academic Press Inc **2** 1 (1953)
8 Schneiderman H A and C M Williams *Biol Bull* **105** 320 (1953)
9 Harrison R G *Harvey Lectures 1933–34* **29** 116 (1935)
10 Weiss P and J H Overton *Excerpta Medica* **8** 424 (1954) Overton J H (in press)
11 Walker P M B *J Exp Biol* **31** 8 (1954)
12 Weiss P *Growth Symposium* (in press)
13 Weiss P and F Rossetti *Proc Nat Acad Sci* **37** 540 (1951)
14 Kollros J J *Physiol Zool* **16** 269 (1943)

2

Methods of Detection and Assay of Growth Hormone*

Jane A Russell
Division of Basic Sciences in the Health Services Emory University Georgia

To demonstrate that growth hormone the subject of this symposium exists at all to define it as a biological or chemical entity we must first consider what is meant by growth What is the nature of this process that it can be affected by growth hormone? Then the changes expected must be defined so that they may legitimately be measured The previous speaker has introduced us to the first consideration To summarize this matter concisely although perhaps not completely one may say that for growth to occur there must be an accretion of material similar in composition to that originally present in the organism An increase in mass alone may not be a sufficiently specific criterion although we are accustomed to think of this as growth For example the addition of either water or fat alone would not constitute true growth in most animal structures There must be a synthesis of protoplasm with its characteristic proteins and nucleic acids with its salts and water in appropriate proportions together with smaller amounts of other indispensable compounds Since an animal is a complex organization of specialized tissues rather than a bag of homogeneous protoplasm all parts of the body may not be affected equally and specific alterations may occur in some tissues in accordance with function in the general growth process Growth then is the sum of many changes in the quantity of organized material and it is a measure of this end effect which we seek

It follows that an alteration in the content of almost any cellular constituent might conceivably be used as an index of the activity of growth hormone in animals For such a criterion to be reliable however the effect must be representative of or a step in the more general action of synthesis of new cellular material With these considerations implicit tests for growth

* Supported by a grant in aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council

diversified cell populations It seems hardly necessary to labor the cautionary lesson contained in this example

To sum up what we measure as growth is the resultant of a heterogeneous array of processes of most diverse kinds and there is no way of telling in advance without careful analysis just what component may be affected by any particular agent in any particular tissue in any particular species and how As long as we keep this in mind we are on safe ground even if for convenience we resort to such shorthand reference terms as the one heading this conference Even if the growth hormone' should turn out not to be just what its name implies I doubt whether this would in the least detract from the superb factual achievements in its study of which we are to hear an up to date account just as I doubt that progress in X rays would have been delayed if they had instead been named death rays because undeniably they sometimes kill I also have little doubt that those with firsthand experience in this field are essentially aware of what the real situation is and how complex it is Yet, there are also those whom the simple label growth might delude into holding complacent oversimplifi and unrealistic notions of its content My comments were intended to restore the complex problem to plain view—not for discouragement but simply for clarification of the problems so that research may orient itself toward the real thing instead of to a verbal symbol As for my final answer to What is Growth? I am tempted to dodge by saying Let us go back to work and find out more about it and not pretend we know

References

1 Weiss P *Chemistry and Physiology of Growth* Ed A K Parpart Princeton University Press 1949 135
2 Hamburger V and R Levi Montalcini *Genetic Neurology* Ed P Weiss University of Chicago Press 1950 128
3 Caspersson T O *Cell Growth and Cell Function* New York W W Norton & Co Inc 1950 185
4 Weiss P and H B Hiscoe *J Exp Zool* **107** 315 (1948)
5 Fankhauser G *Quart Rev Biol* **20** 20 (1945)
6 Schrader F and C Leuchtenberger *Exp Cell Research* **1** 421 (1950)
7 Swift H H *International Review of Cytology* Eds Bourne G H and J F Danielli New York Academic Press Inc **2** 1 (1953)
8 Schneiderman H A and C M Williams *Biol Bull* **105** 320 (1953)
9 Harrison R G *Harvey Lectures 1933–34* **29** 116 (1935)
10 Weiss P and J H Overton *Excerpta Medica* **8** 424 (1954) Overton J H (in press)
11 Walker P M B *J Exp Biol* **31** 8 (1954)
12 Weiss P *Growth Symposium* (in press)
13 Weiss P and F Rossetti *Proc Nat Acad Sci* **37** 540 (1951)
14 Kollros J J *Physiol Zool* **16** 269 (1943)

Table I

METHODS FOR THE DETECTION OR ASSAY OF GROWTH HORMONE

Test	*Duration of Treatment*	*Precision* (λ)*
A Body Size		
Body weight increase		
Mature intact female rat	15–20 days	2–3
Hypophysectomized rat	10–14 days	3–4
Dwarf mouse	14 days	2–7
Tail length increase (hypox rat)	7–14 days	2–5
Tibial epiphysis increase in width in hypox rat	4 days	3
Organ weight (e g liver thymus) increase	4–14 days	*
B Metabolism of Nitrogen Phosphorus or Sulfur		
Nitrogen balance intact dog or rat	1–5 days	*
Plasma amino nitrogen decrease	2–6 hours	4
Blood urea decrease	2–6 hours	—
Urea formation after protein hydrolysate	1–3 hours	2–6
Tissue constituents (e g amino nitrogen amide nitrogen glutathione)	varied	—
Tissue enzymes (e g transaminases) increase or decrease	7–14 days	—
N^{15} retention	2 days	—
Plasma phosphate increase (hypox rat)	15 days	*
Plasma or tibial phosphatase increase (hypox rat)	15 days	*
Uptake of S methionine into muscle protein	3 days	—
C Carbohydrate Metabolism		
Muscle or cardiac glycogen maintenance in fasting hypox rat	24 hours	6
Cardiac glycogen increase in intact rat	6–12 hours	8
R Q depression in fed intact rat	2–6 hours	*
Diabetogenic action intact rat	4 days	*

* Significant relationship between response and dose demonstrated Index of precision (λ = stand dev in terms of log dose) given if estimate available

factors than growth hormone Thyrotropic hormone like thyroxin may augment the action of growth hormone in such preparations while adrenocorticotropic hormone in any considerable amount will greatly depress the response That the intact rat is more sensitive or less sensitive than the hypophysectomized animal to these agents has not been demonstrated In both types of animals non specific effects such as those affecting appetite and water retention for example may influence the response to growth hormone Thus although measurement of change in body weight is the most simple procedure available a method of assay based on this device has disadvantages it takes weeks to complete it requires a fair amount of material and most important it is not completely satisfactory as to specificity

hormone action have been confined principally to two types of observations In the first the induction of a sufficient increase in size of the body or of a constituent part will leave no doubt that true growth has taken place *Under certain conditions increases in body weight* or in an organ dimension such as the tail length or the tibia epiphyseal width, have been taken as representative of the rate of growth In the second type of observation attempts have been made to obtain an indication of change in the rate of protein synthesis This is perhaps a more specific response, at least in theory for there is no substance more characteristic of animal tissues than protein and retention of nitrogen in this form is indubitably an indication of growth of some part of the organism Direct measurement of the rate of this reaction is difficult however so that indirect effects on nitrogen metabolism have been used and these may not strictly relate to growth

At best, these and similar methods of detecting growth hormone activity *are likely to be faulty Only one aspect of growth* can usually be measured at a time and what is observed is always a resultant of many actions bearing directly or indirectly on the growth process The ideal method would be one in which measurement was made of the rate of a single metabolic reaction controlled directly by growth hormone but as we all know this is not yet possible

The observed effects of growth hormone are so multifarious that it is possible to make a considerable list of procedures which might be employed for the detection or assay of the hormone Many of these have been described in the review of Li and Evans[1] and in the article of Greenspan Li Simpson and Evans in Emmen s *Hormone Assay* In Table 1 are listed the principal items together with the usual duration of the test and an indication of the precision of the assay where this is known

The methods based on changes in body weight have been the ones most commonly employed to date With the use of standardized animals an adequate diet and a sufficiently long term of treatment the method can be made reasonably precise either in mature intact female rats or in young hypophysectomized rats The latter animals are more economical for several reasons They weigh much less the gain in weight which can be measured accurately is smaller and the treatment period is usually somewhat shorter The hypophysectomized animal is said also to be much more sensitive to growth hormone than is the intact animal but if the dosage is adjusted to body weight the reported data do not indicate any very great difference in this respect The use of the hypophysectomized animal has been favored for this test not only because of the smaller amount of hormone required but mainly because *many workers have thought that the* growth response was more specific than that in the intact animal Even in the hypophysectomized rat however it is certain that the response to administered pituitary preparations can be greatly affected by the presence of other

muscle glycogen of fasting hypophysectomized rats and in hastening and augmenting the increase in cardiac glycogen which occurs in intact animals during fasting All these effects may be regarded perhaps as expressions of a single growth hormone action on the utilization of carbohydrate If they represent another facet of growth hormone activity then they could be used as methods for assaying the hormone Until there are stronger theoretical reasons for associating the effects on carbohydrate metabolism with nitrogen retention and growth there must always be some reservations as to this identity An additional complication arises moreover in that adrenocortical factors appear to be permissive or synergistic in many of the actions of the pituitary factor on carbohydrate metabolism Hence present assays of activity in respect to this class of effects although they may furnish corollary information of much interest probably are not equivalent to assays of growth hormone by more conventional tests

With respect to the effects of growth hormone on nitrogen metabolism the two which I wish to discuss further are the depression in circulating amino nitrogen in intact rats and the diminution in urea formation after the administration of protein hydrolysate to nephrectomized rats In the normal rat the administration of a single dose of growth hormone is followed by a drop of 20 to 30 per cent in the blood or plasma amino nitrogen content the level reaching a minimum in about 4 to 6 hours Since the effect is reasonably interpreted as being a result of growth hormone action on nitrogen retention and since the procedure is simple and very quick a method of assay based on this response could be advantageous We have found in tests of this procedure as have others [7] that it is not very sensitive the minimum effective dose being about 1 mg per 100 g body weight The ranges of effective dosage and of response are both short There is little gradation between barely significant and maximal effect while the variation in response is large compared with its range (Table 2) On one occasion we did obtain a significant dose response relationship with an 0 4 index of precision Hence it is possible that an assay method acceptable for certain purposes at least could be devised on this basis On the other hand it must be recalled that a depression of plasma amino nitrogen content is not specifically a response to growth hormone This effect is induced by epinephrine or agents causing its secretion by insulin or by glucose and it may be seen as well after some forms of cortical hormone Accordingly this lack of specificity might preclude the use of this procedure for general assay purposes even if it could be made as quantitative as desired

The other method mentioned above is one to which more attention has been devoted In studies of nitrogen metabolism and of nitrogen balance it has always been difficult to make accurate observations of rates of nitrogen excretion over periods of less than a day and often several days are required for an acceptable precision As was shown by Engel and collaborators [8] however the rate of increase in blood urea in nephrectomized rats affords

Other procedures utilizing effects of growth hormone on body size have not been so much used The dwarf mouse as a subject has the inconvenience of rarity and moreover the specificity of the response in this animal is questionable[3,4] Measurement of the tail length in hypophysectomized rats has appeared to offer a reasonably satisfactory approach at least in certain strains of rats, but published data on the method are few It has the disadvantage of requiring the use of hypophysectomized animals in the immediate post operative period and the specificity of this method also has not been established

Increase in the width of the tibial epiphysis of the hypophysectomized rat has been found by Greenspan et al[5] to furnish the basis of one of the better assays for growth hormone activity The details of this procedure and factors affecting it are the subject of another paper in this symposium It may be said here that the method has the advantages of high sensitivity comparative precision and relatively short duration of treatment However it requires a rather time consuming technique while the base line values and responses vary considerably from time to time This requires rigid care in standardizing the experimental conditions and here too the response is much affected by pituitary factors other than growth hormone which may be present in the preparations to be tested

The other principal group of observations which might be used for assay purposes concern a variety of effects of growth hormone on the metabolism of nitrogen phosphorus and sulfur (see Table 1) In general these have not been studied as extensively as the procedures previously described It is known that a significant relationship between response and dosage of growth hormone may be obtained in some cases but there have been few studies of the degree of precision afforded of specificity or of sensitivity to extraneous factors In other instances it is not possible to say more than that a measurable effect of growth hormone occurs It is quite conceivable that one or more of these effects not yet studied in detail might provide the basis of a suitable assay method On two of these responses we have obtained further information which will be presented shortly

The possibilities of assays based on a third group of effects may also be mentioned These concern the actions of pituitary preparations on some aspects of carbohydrate metabolism exemplified by various forms of glycostatic activity depression of the R Q in fed animals and diabetogenic effects To date activity in these respects has always accompanied growth hormone during its isolation and purification and no separation of these effects from those on growth or nitrogen retention has yet been achieved The extensive and detailed observations of Reid[6] indicate a high degree of parallelism between the growth promoting potencies in intact rats and the diabetogenic activities in cats when a number of variously treated growth hormone preparations were studied A similar trend was seen in our own observations on the action of growth hormone both in maintaining the

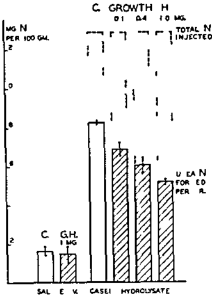


Fig 1 The effect of growth hormone on the rate of urea formation during the first hour after the injection of saline or of an enzymatic casein hydrolysate in fasting rats Columns outlined in solid lines represent urea accumulation in mg nitrogen per 100 g of body weight those in broken lines the amount of nitrogen injected as hydrolysate (From Russell *Endocrinology* 49 99 1951)

have been assayed by this procedure The conditions currently employed are as follows young adult rats fasted for 24 hours are nephrecto mized under ether anesthesia and allowed to recover for a few hours The preparation to be tested is given intraperitoneally in a small volume of saline 1 to 1½ hours before measurements of blood urea are begun A sample of blood is drawn from the tail a measured amount of casein hydrol ysate (Parenamine—Winthrop–Stearns—1 ml containing 12 to 14 mg N per 100 g body weight) is injected intravenously over a period of a few minutes and a blood sample is taken again 2 hours later The increase in blood urea nitrogen is determined in series of 4 to 6 animals at a time With rats of the same size and age and with a standardized technique the increase in blood urea N is fairly constant averaging about 22 to 25 mg percent with a standard deviation of 1 to 2 mg per cent With large doses of growth hormone the 2 hour increase in blood urea may be reduced to 12 to 14 mg per cent

The relationship between dosage and response and the precision of the method as obtained with several different growth hormone preparations are presented in Figure 2 Since these assays were done at different times the control values varied somewhat Accordingly small differences in position of the assay curves are not necessarily significant The effect is seen to be reasonably reproducible but it must be admitted that the accuracy of the

Table 2

EFFECT OF GROWTH HORMONE ON BLOOD AMINO NITROGEN IN FASTING RATS

	No of Obs	Blood Amino Nitrogen mg per cent	
		Initial	Change in 5 Hours
A Saline	4	11 5	−0 7 ± 0 2
B Growth Hormone			
0 5 mg per 100 g	4	10 8	−1 0*
1 0	4	11 4	−2 2*
2 0	4	11 6	−2 5*
5 0	5	11 6	−2 9*
10 0	4	12 4	−2 3

* Standard deviation within groups or from regression 0 75 mg per cent b = 1 8 mg per cent per log unit λ = 0 42

a reproducible means of following the rate of nitrogen metabolism in experiments of short duration as little as 1 to 3 hours being sufficient for accurate observations Since growth hormone was known to depress the levels of blood amino nitrogen and of urea in a short period of time it appeared possible that nitrogen retention after growth hormone might also be observed in similar circumstances if the technique of Engel et al was employed With this procedure it was found that if growth hormone was given to nephrectomized rats in a basal state little or no effect on the rate of urea formation was seen in the next few hours However when an amino acid mixture was given to the animal and the rate of urea formation thus greatly enhanced a nitrogen retaining effect of growth hormone was clearly seen[9] This response was taken to be the result of an increase in the uptake of amino acids into protein rather than any direct effect on the rate of catabolism of the amino acids or on the rate of urea formation per se for it was accompanied by enhancement of the rate of removal of amino acids from the blood as well as by diminished concentrations of free amino and amide nitrogen in the tissues The effect is thus reasonably interpreted as part of the growth process

This acute response to growth hormone in respect to nitrogen retention was first demonstrated with an active but impure preparation of the hormone Later when highly purified materials were available it was shown that the reaction was fairly sensitive 100 μg per 100 g or less sufficing for a significant depression in the rate of urea formation (Fig 1) Moreover the response was related to dosage with an encouraging degree of precision[10] (λ, 0 4) Further investigations have now been made of a number of factors concerned in the response and several growth hormone preparations

hormone on nitrogen metabolism there is no reason to expect an augmentation of growth hormone action by thyrotropin within the few hours allowed for the response Moreover, since the observations are made in intact rats rather than in hypophysectomized animals it is questionable whether any enhancement by thyrotropin could be expected in any case Confirmation of these suppositions is desirable but we have not obtained the purified sample of thyrotropic hormone certainly free of growth hormone which would be required for this experiment

A more serious possibility is that adrenocortical steroids secreted under the influence of corticotropin might increase the catabolism of protein and so interfere with observations of the nitrogen retaining action of growth hormone The observations of Engel and co workers[11 1] indicate that this would not be expected under the conditions of the test In their work cortical hormones were found to increase urea formation in the fasting animal only after a latent period of 3 hours or more while the catabolic effect was not evident after administration of amino acid mixtures Employing the conditions of the assay for growth hormone described above we have given a commercial protein corticotropin preparation in place of growth hormone and also with growth hormone in the same solution At the moderately large dosage of ACTH used (1 unit per 100 g body weight) no effect of this hormone on the rate of urea formation was seen in either case (Fig 3) In view of Engel s observations and our own it appears that the duration of this test for nitrogen retention is too short for it to be affected by any concomitant adrenocortical stimulation This is perhaps the greatest advantage of this method of assay of growth hormone activity

The observations presented thus far indicate that measurement of the rate of urea formation after amino acid administration affords a rapid specific and moderately precise method for the assay of nitrogen retaining factors found in the anterior pituitary and that the property of this response accompanies growth hormone during its isolation and purification A question may still be raised concerning the relationship of the activity measured in this test to that obtained by more usual methods of measuring growth promotion Precise estimates of potency ratios of various growth hormone preparations by the several methods have not been made for the errors of the methods are such as to make this a most laborious undertaking Until recently we had been satisfied to find that all the purified growth hormone preparations so far tested brought about an acute retention of nitrogen and that only materials active by the body weight or tibial assays were effective here Now our assurance in this respect has been somewhat disturbed Through the courtesy of Doctors Raben and Astwood we were supplied with some pituitary preparations made by the oxycel adsorption procedure which were highly potent as ACTH and which were also found by Dr Raben to be active in respect to certain effects on carbohydrate metabolism [13 14] By the usual biological tests and from their chemical prop

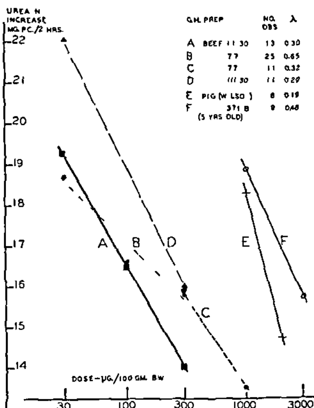


FIG 2 The relationship between dose of growth hormone and rate of urea formation after casein hydrolysate in fasting rats

method as a quantitative assay still is not as great as would be desirable The precision of the assay appears limited to a large extent by the range of responses which can be observed If the maximal effect of the hormone could be enhanced as perhaps by changes in the mixture of amino acids employed or in their method of administration it is possible that the accuracy of the method could be improved but we have as yet little information on these points

Numerous tests have been made with different proteins and with inactivated hormone preparations and results indicate no significant effect on urea formation Neither epinephrine nor insulin in moderately large amounts (05 *mg* or 0 5 unit per *kg* respectively) altered the rate of urea formation appreciably either in the presence or in the absence of exogenous amino acids

A question of much importance is of course whether or not the presence of thyrotropic or adrenotropic hormones in this test may affect the response obtained from pituitary preparations From the known effects of thyroid

ment of purified growth hormone converted it from a substance affecting both body size and nitrogen metabolism to one affecting only the latter function would the second substance still be a growth hormone?

Summary

The theoretical basis of methods for the detection and assay of growth hormone activity was discussed and the available practical procedures re viewed briefly The best of these methods were considered to be deficient in one or more respects inconvenient because of the nature of the test the duration of treatment or amount of material required lacking in specificity or precision or sensitive to the action of extraneous factors A new method of assay based on the diminution in rate of urea formation after exogenous amino acids in nephrectomized rats was described This procedure was found to furnish a rapid specific and moderately precise measure of effects on nitrogen retention and was not affected by the presence of adrenocortico-tropic hormone Purified growth hormone was highly active in this respect but the precise relationship of nitrogen retaining activity as judged by this procedure to the promotion of growth as judged by effects on body size is not yet certain

References

1 Li C H and H M Evans *Recent Progress in Hormone Research* 3 1 (1948)
2 Greenspan F S Li C H Simpson M E and H M Evans *Hormone Assay* Ed C W Emmens New York Academic Press 1950 273
3 Bates R W Laanes T and O Riddle *Proc Soc Exp Biol Med* 33 446 (1935)
4 Kemp T and L Marx *Acta Pathol Scand* 13 512 (1936) 14 197 (1937)
5 Greenspan F S Li C H Simpson M E and H M Evans *Endocrinology* 45 455 (1949)
6 Reid E *J Endocrinology* 8 50 (1952)
7 Reid E *J Endocrinology* 9 329 (1953)
8 Engel F L Pentz E I and M G Engel *J Biol Chem* 174 99 (1948)
9 Russell J A and M Cappiello *Endocrinology* 44 333 (1949)
10 Russell J A *Endocrinology* 49 99 (1951)
11 Engel F L Schiller S and E I Pentz *Endocrinology* 44 458 (1949)
12 Engel F L *Endocrinology* 50 462 (1952)
13 Astwood E B Raben M S Payne R W and A B Grady *J Am Chem Soc* 73 2969 (1951)
14 Westermeyer V W and M S Raben *Endocrinology* 54 173 (1954)

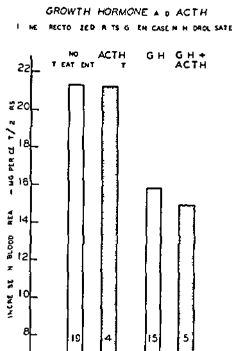


FIG 3 Effect of growth hormone (0 3 mg per 100 g) and adrenocortico tropic hormone (1 unit of protein hormone per 100 g) on urea formation after casein hydrolysate in nephrectomized rats Dots at top of column indicate standard error figure at bottom of column indicates number of observations per group From Rundell and Russell (unpublished)

erties these preparations were not considered to contain growth hormone Although highly active as corticotropin these preparations did not increase nitrogen catabolism and surprisingly were quite effective in suppressing urea formation in nephrectomized rats Furthermore it was found that when this oxycel material or in one instance purified growth hormone, was subjected to mild treatment with alkali a procedure which inactivates growth hormone as judged by body weight assays the effect on urea formation persisted

These observations now raise some interesting possibilities One is that the factor responsible for the acute effect on nitrogen metabolism although usually accompanying growth hormone is separable from it The other is that there is such a thing as a degraded growth hormone one which is active in tests of short duration but which is incapable of promoting observable growth when given once a day in chronic tests of the usual type A smaller molecule than protein growth hormone, one which is rapidly absorbed and destroyed might conceivably behave in this way As yet it is not possible to decide between these possibilities Here we return again to the questions of what is growth and what constitutes a valid measure of hormonal action on growth If it should be proved true that chemical treat

tilage plates of these older animals The following year Ingalls [8] and Ingalls and Hayes [9] simultaneously with Ray Evans and Becks [10] reported on the development of the proximal epiphysis of the tibia in normal and hypophysectomized rats The latter group ascribed the reduction in the width of the epiphyseal cartilage which followed hypophysectomy to a disturbance in the equilibrium established between the formation of cartilage and bone in endochondral ossification Shortly thereafter Becks Kibrick, Marx and Evans[11] extended the observations of Ray et al to the very early effects of hypophysectomy (4 days after operation) Very detailed reports on the changes with age in the histological appearance of the proximal tibial epiphysis of the normal female rat and of the female rat hypophysectomized at 26 days of age have been presented by Becks, Simpson and Evans [1, 13] Figure 1 has been drawn on the basis of data compiled by these investigators and demonstrates the very rapid narrowing of the cartilage plate which occurs between days 40 and 65 The rate of decrease in width after the 65th day is much slower Figure 1 also illustrates the very rapid decrease observed in the experimental animal following hypophysectomy By the 14th postoperative day the width of the disc has essentially become constant, this stasis finally resulting in a wider plate in the older hypophysectomized animal than in a normal animal of the same age

Effect of Ovariectomy The precipitous fall during the period between the 40th and 65th day occurs at the time of beginning sexual maturity in these animals and is reflected in a marked decrease in the rate of growth of the tibia Furthermore as shown first by Gaarenstroom and Levie[13] and sub-

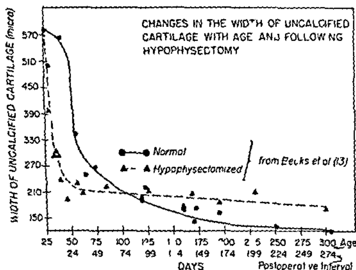


Fig 1 The change in the width of the tibial proximal epiphyseal cartilage of the female rat (Long Evans) with age and following hypophysectomy at 26 days of age From Becks et al [13]

3

The Tibia Test for Growth Hormone*

Irving I Geschwind and Choh Hao Li
Hormone Research Laboratory University of California Berkeley

Introduction

Pituitary Control of Proximal Tibial Epiphysis In 1923 Dott and Fraser[1] reported that one sequel of hypophysectomy in cats and dogs was a generalized decrease of epiphyseal activity in the long bones in a separate report Dott reported an increased activity following the feeding of desiccated anterior lobe of the pituitary gland Seven years later, Handelsman and Gordon[3] observed growth of the skull and mandible in rats receiving injections of alkaline pituitary extracts Renewed interest in the action of pituitary extracts on the epiphyses of the long bones arose from the work of Lucke and Huckel[4] in 1933 and Silberberg[5] in 1935 The former workers reported that administration of such extracts to 6 week old rats resulted in a marked increase in the width of the epiphyseal cartilage of the tibia while Silberberg noted that as few as 4 injections of an acid extract of the anterior pituitary were capable of stimulating growth of tibial cartilage and bone in the normal young guinea pig In 1939 Freud Levie and Kroon[6] investigated the effects of hypophysectomy and of maintenance with growth hormone concentrate on the development of the vertebrae ribs and tibias of the rat From the results of this investigation they concluded that growth hormone has a biologically typical point of attack at the proliferating cartilage Up to 1940 the major part of the previous work had been concerned with the action of growth hormone in young animals however in that year Ross and McLean[7] administered growth hormone to weight plateaued rats and observed a reawakening of active growth in the quiescent epiphyseal car

* This work is supported in part by grants from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council and the Albert and Mary Lasker Foundation Grateful acknowledgement is made to Charles Jordan and Harold Papkoff for able technical assistance

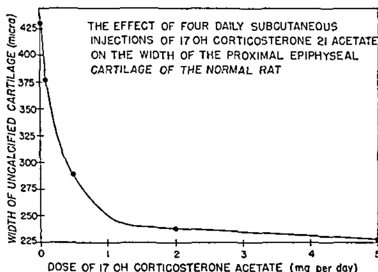


FIG 2 The narrowing of the epiphyseal cartilage of the normal rat by graded doses of hydrocortisone administered over a period of 4 days

As an example Becks et al [13b] found the tibia cartilage width of normal 56 day old male rats to be 409 micra whereas an average value of 294 micra was found for animals of the same age which had been adrenalectomized 30 days previously These latter animals maintained on 1% NaCl manifested a satisfactory weight gain and good skeletal growth

The effect on the normal cartilage disc width of administration of the adrenocortical steroid 17 hydroxycorticosterone has recently been investigated in this laboratory The results of this investigation in which graded doses of Compound F were given for 4 days to 27 day old normal female rats is shown in Figure 2 Subcutaneous injections of as little as 100 micrograms per day of the steroid resulted in a significant narrowing of the cartilage plate and a maximal response was obtained with approximately 2 mg per day In an attempt to counterbalance the effect of the administered steroid different groups of animals were injected with graded doses of growth hormone along with either 0 5 or 2 mg per day of the steroid It was found however that even with the smaller quantity of Compound F co administration of large doses of growth hormone (2 5 mg per day) could not increase the width of the disc more than 80 micra so that the maximally widened disc was still narrower by 100 micra than that found in normal uninjected animals of the same age The results of these experiments are typical of the antagonism of hypercorticalism to normal growth patterns and responses

Effect of Thyroid Another endocrine factor concerned in the regulation of the width of the normal disc is represented by the thyroid and its secretion The marked failure of the disc in the thyroidectomized animal to

sequently confirmed by many workers, estrinization of the young rat produces a shrinkage of the epiphyseal cartilage In order to determine the extent of influence of beginning estrogen secretion on the width of the epiphyseal plate we have recently investigated the effect of ovariectomy on the width of the uncalcified cartilage of the tibia A group of rats (Long Evans strain) was ovariectomized between days 28 and 35 they were then autopsied at various postoperative intervals up to day 64 The results of *these experiments (Table 1) demonstrate that approximately one half of* this rapid decline with age may be prevented by ovariectomy At day 38 eight days following gonadectomy no effect is observed which may be ascribed to the operative procedure which is in keeping with data indicating this age to be just prior to or to mark the beginning of sexual function in rats The table also indicates that the rate of fall of disc width in intact male rats is comparable to that in ovariectomized rats and that orchiectomy tends to produce a further narrowing of the disc though with the limited number of animals employed the differences are not significant

Table 1

THE CHANGE WITH AGE IN THE WIDTH OF THE PROXIMAL EPIPHYSEAL CARTILAGE OF THE TIBIA OF THE NORMAL AND CASTRATED RAT

*Sex**	*Age (days)*	*Oper Cond'n*	*Post Oper Interval (days)*	*No of Animals*	*Width of Uncalified Cartilage Plate (micra)*
F	30–31	—	—	11	442 (368–535)†
F	34–40	—	—	6	397 (362–448)
F	42	—	—	6	347 (311–420)
F	56–62	—	—	15	260 (238–282)
F	65	—	—	4	230 (218–252)
F	38	Ovar	8	3	409 (374–441)
F	56	Ovar	21	13	364 (331–426)
F	64	Ovar	36	18	314 (280–346)
M	29	—	—	5	503 (484–524)
M	36	—	—	5	477 (441–510)
M	59	—	—	6	324 (298–344)
M	59	Castr	30	6	283 (247–324)

* F = female M = male
† Range of responses in parentheses

Effect of Adrenal Cortex Another factor probably operative at all times is represented by the steroids secreted by the adrenal cortex A marked reduction in the width of the epiphyseal cartilage following the administration of ACTH to normal animals has been reported by Becks Simpson Li and Evans [13b] However *in all probability some secretion from* the adrenal cortex is required for the maintenance of the normally wide disc found in young animals as is evidenced by studies in the adrenalectomized animal

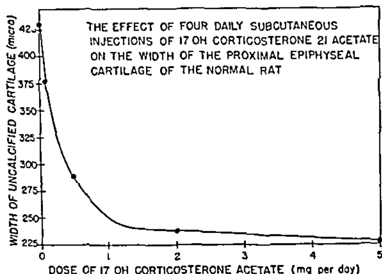


FIG 2 The narrowing of the epiphyseal cartilage of the normal rat by graded doses of hydrocortisone administered over a period of 4 days

As an example Becks et al[13b] found the tibia cartilage width of normal 56-day old male rats to be 409 micra whereas an average value of 294 micra was found for animals of the same age which had been adrenalectomized 30 days previously These latter animals maintained on 1% NaCl manifested a satisfactory weight gain and good skeletal growth

The effect on the normal cartilage disc width of administration of the adrenocortical steroid 17 hydroxycorticosterone has recently been investigated in this laboratory The results of this investigation in which graded doses of Compound F were given for 4 days to 27 day old normal female rats is shown in Figure 2 Subcutaneous injections of as little as 100 micrograms per day of the steroid resulted in a significant narrowing of the cartilage plate and a maximal response was obtained with approximately 2 mg per day In an attempt to counterbalance the effect of the administered steroid different groups of animals were injected with graded doses of growth hormone along with either 0 5 or 2 mg per day of the steroid It was found however that even with the smaller quantity of Compound F co-administration of large doses of growth hormone (2 5 mg per day) could not increase the width of the disc more than 80 micra so that the maximally widened disc was still narrower by 100 micra than that found in normal uninjected animals of the same age The results of these experiments are typical of the antagonism of hypercorticalism to normal growth patterns and responses

Effect of Thyroid Another endocrine factor concerned in the regulation of the width of the normal disc is represented by the thyroid and its secretion The marked failure of the disc in the thyroidectomized animal to

sequently confirmed by many workers estrinization of the young rat produces a shrinkage of the epiphyseal cartilage In order to determine the extent of influence of beginning estrogen secretion on the width of the epiphyseal plate we have recently investigated the effect of ovariectomy on the width of the uncalcified cartilage of the tibia A group of rats (Long Evans strain) was ovariectomized between days 28 and 35, they were then autopsied at various postoperative intervals up to day 64 The results of these experiments (Table 1) demonstrate that approximately one half of this rapid decline with age may be prevented by ovariectomy At day 38 eight days following gonadectomy no effect is observed which may be ascribed to the operative procedure which is in keeping with data indicating this age to be just prior to or to mark the beginning of sexual function in rats The table also indicates that the rate of fall of disc width in intact male rats is comparable to that in ovariectomized rats and that orchiectomy tends to produce a further narrowing of the disc though with the limited number of animals employed the differences are not significant

Table 1

THE CHANGE WITH AGE IN THE WIDTH OF THE PROXIMAL EPIPHYSEAL CARTILAGE OF THE TIBIA OF THE NORMAL AND CASTRATED RAT

*Sex**	*Age (days)*	*Oper Cond'n*	*Post Oper Interval (days)*	*No of Animals*	*Width of Uncalcified Cartilage Plate (micra)*
F	30–31	—	—	11	442 (368–535)†
F	34–40	—	—	6	397 (362–448)
F	42	—	—	6	347 (311–420)
F	56–62	—	—	15	260 (238–287)
F	65	—	—	4	230 (218–252)
F	38	Ovar	8	3	409 (374–441)
F	56	Ovar	21	13	364 (331–476)
F	64	Ovar	36	18	314 (280–346)
M	29	—	—	5	503 (484–524)
M	36	—	—	5	477 (441–510)
M	59	—	—	6	324 (298–344)
M	59	Castr	30	6	283 (247–324)

* F = female M = male
† Range of responses in parentheses

Effect of Adrenal Cortex Another factor probably operative at all times is represented by the steroids secreted by the adrenal cortex A marked reduction in the width of the epiphyseal cartilage following the administration of ACTH to normal animals has been reported by Becks Simpson Li and Evans [13b] However in all probability some secretion from the adrenal cortex is required for the maintenance of the normally wide disc found in young animals, as is evidenced by studies in the adrenalectomized animal

injection with growth hormone and that beginning on the sixth day a level ing of the response was obtained hence the growth hormone preparation was injected daily for only 4 days The experimental animals were 38 day old hypophysectomized female rats and the series of injections was begun 12 days after operation The responses which were obtained when graded doses of the hormone were administered fell on a straight line when plotted against the logarithm of the doses (Fig 3)

Though this test proved to have far greater sensitivity and much less variability than the other tests available the time consumed in preparing serial sections of bone and in staining these sections negated to a great extent the advantages of the method and militated against its routine use However after the above report was submitted early in 1943 Evans Simpson Marx and Kibrick[15] presented a modification which would permit its use for routine assays This modification made use of silver nitrate to stain the calcified portion of bone halves and thus demarcate the epiphyseal disc Briefly the method is as follows [1 16]

Assay Procedure Female rats are hypophysectomized at 26 to 28 days of age and following a postoperative interval of 12–14 days administration of growth hormone is begun The hormone in aqueous solution is injected intraperitoneally once daily for four days Twenty four hours after the last injection the animals are sacrificed a tibia is dissected free of the accompanying soft tissue and then split at the proximal end in the mid sagittal plane The bone halves may then be stained with silver nitrate or fixed in 10% neutral formalin If they are to be stained immediately the following modification of the suggested procedure is employed The bone halves are washed in water for 10 minutes immersed in acetone for about 6 minutes and then washed in water again for 3 minutes They are then placed in freshly prepared 2% silver nitrate for approximately 2 minutes and then rinsed in water While under water the bone halves are exposed to a strong light and when the calcified portions become dark brown they are removed to a microscope stage and the width of the uncalcified epiphyseal cartilage is measured under low power using a calibrated micrometer eye piece From 8 to 10 individual readings are made across the epiphysis This simplified procedure for staining is much less time consuming than the original method and has been thoroughly cross-checked with it to insure accuracy and dependability It has been employed routinely in this labora tory for the past five years

The dose response curve obtained when this procedure was followed using a preparation of growth hormone similar to that used by Kibrick et al [14] is presented in Figure 3 and is taken from the data compiled by Marx Simpson and Evans [17] To be noted are (a) the straight line relation ship between the response and the logarithm of the dose (b) the similarity of the slope of the curve obtained from the data of Marx et al [17] to that obtained by Kibrick et al [14] and (c) the much lower value obtained for

narrow normally with age[1] is one of the manifestations of the lack of differentiation which occurs following this operation

The Tibia Test

Subsequent to the experiments of Freud Levie and Kroon[6] who employed purified preparations of growth hormone both Ray Evans and Becks[10] and Becks Kibrick Marx and Evans[11] reported on the effects obtained following the administration to normal[10] and hypophysectomized[10,11] animals of growth hormone preparations then being prepared in the Institute for Experimental Biology at Berkeley The first attempt, however to study the relationship between the dose of growth hormone and the extent to which bone growth was stimulated is to be found in the report of Kibrick Becks Marx and Evans[14] The index of activity which was selected was the increase in the width of the proximal epiphyseal cartilage of the tibia as measured in a histological preparation with an eyepiece micrometer It is of interest to quote in toto the premises which formed the basis for this choice

> Hypophysectomy rapidly initiates a loss in the dimensions of the epiphyseal cartilage plate despite the fact that growth of cartilage and bone may continue for a short time in the young animal after the removal of the pituitary This loss in thickness reflects the initial disturbance of the equilibrium that normally exists between chondrogenesis and osteogenesis Administration of growth hormone rapidly restores the dimensions of the cartilage plate by stimulating first chondrogenesis and then osteogenesis until an equilibrium is reestablished[14]

Preliminary experiments by these authors revealed that a rapid increase in the width of the cartilage could be expected up to the fourth day of

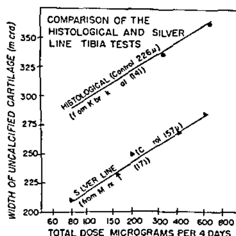


FIG 3 Dose response curves for similar growth hormone preparations The upper curve was determined by Kibrick et al[14] who measured the response in stained histological sections of bone the lower from Marx et al[17] employing the silver nitrate reaction on bone halves

of the standard curve as an absolute standard is statistically questionable In order to obtain results capable of statistical evaluation both the un known preparation and a laboratory standard preparation (L2518B) have been subjected to a triple dose assay procedure similar to that employed by Sayers Sayers and Woodbury[10] for the assay of ACTH With this method groups of 4 rats are injected with one of three doses of either the unknown or the standard the total doses of the standard amount to 20 60 and 200 micrograms while those of the unknown are adjusted to give responses in the same range as those given by the standard As an example Table 2 records the statistical values* calculated from the results of assays performed on four separate occasions over a period of three months of the "core material resulting from a partial chymotryptic hydrolysis of growth hormone [9] The ratio of potency of the unknown (the core Prep L2552A) to standard the standard error of the potency the range of potencies that may be expected in 95 out of 100 cases the combined slope b and the derived value λ (= s/b)— the index of precision are given for each assay The consistency of the calculated potencies as well as the excellent values of λ attest the statistical adequacy and accuracy of the assay procedure

Table 2

THE REPEATED ASSAY OF A GROWTH HORMONE PREPARATION OF UNKNOWN POTENCY

Date	Potency ± S E * (%)	Range of Potency† (%)	Slope (b)	Index of Precision (λ)
7–23	105 3 ± 27 1	64–174	50 8	0 205
7–30	97 5 ± 16 5	70–136	44 5	0 168
8–13	84 9 ± 10 0	67–107	70 3	0 126
9–10	91 8 ± 12 5	70–120	80 6	0 137
	Aver 94 9 ± 16 5		Aver 61 6	0 159

Potency of preparation L2552A compared with that of the standard preparation (= 100%) L2518B

† 95 cases out of 100

Factors Influencing the Assay Procedure

The assay procedure as outlined above is essentially the original one sug gested by Evans et al [15] Certain aspects of this assay method have been re examined in order to determine to what extent variation in or modifica tion of the procedure would still be compatible with meaningful and repro ducible results

* The detailed references for the statistical procedures may be found in ref 19

disc widths of control and injected animals by the silver line method These latter values are of the order of two thirds to three quarters of those obtained by the histological method and are probably due to a staining of the vesicular zone of the cartilage plate by silver nitrate This zone forms the zone of provisional calcification and would react with silver ions

Statistical Treatment of Assay Data The standard curve obtained by Marx et al was reinvestigated by Greenspan Li Simpson and Evans[16] when more highly purified growth hormone preparations became available [18] Figure 4 portrays the standard curve obtained by Greenspan et al and for comparison also presents that obtained by Marx et al A comparison of this sort was previously made by Greenspan et al who calculated that the slopes of the two lines are essentially identical ($b_{Marx} = 82.5$, $b_{G \quad r\,n} = 79.4$) and that the preparation of Li et al was approximately 9 times as potent as that employed by Marx and his co workers Also included is the curve that has been compiled from the data accumulated in this laboratory over the past 4 years It may be seen that there is a considerable difference between our curve and that of Greenspan et al though a substantial number of preparations employed by the latter workers have also been used in establishing the later curve The reason for this discrepancy is not apparent and no satisfactory explanation has so far been found

If a given preparation of growth hormone is repeatedly assayed over a period of weeks and if the response obtained with a given dose is compared with the standard curve variations are found which represent a weekly variation in potency of from 50 to 200 per cent of the mean Thus the use

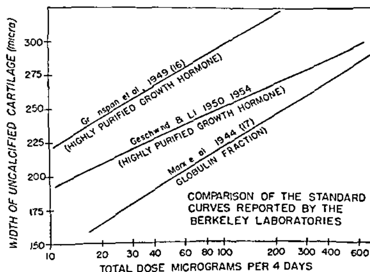


FIG 4 Standard dose response curves for the tibial cartilage response to growth hormone That of Marx et al [17] and of Greenspan et al [16] have been previously reported

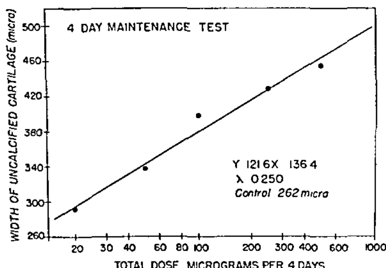


FIG 5 Dose response curve for growth hormone administered for 4 days starting on the day of hypophysectomy (27 days of age) Autopsy was per formed 24 hours after the last injection Silver nitrate reaction on bone halves

received graded doses of growth hormone Injections were given once daily for 4 days and the animals were sacrificed 24 hours after the last injection The results of these experiments are plotted in Figure 5 in the form of a log dose response curve It can be seen that the individual responses fall on a straight line when plotted against the logarithm of the dose Further more the slope of the curve is much steeper than that found in the standard procedure (121 vs 60) The index of precision λ is 0 25 which falls within the acceptable range With the value for the slope b as high as it is the value for λ reflects a moderately large value for the standard deviation of the curve (S) *The high value of S undoubtedly is a result of the two* main disadvantages inherent in this procedure namely the lack of the pre injection postoperative period which ordinarily serves to eliminate those animals which do not fulfill the requirements with respect to weight change noted above and the failure of the dimensions of the cartilage to achieve a constant value prior to the injection period

Diet Adequate control of dietary factors is a *sine qua non* of any assay for growth hormone activity involving growth as an index Some of the dietary factors known to exercise a particular influence on the size of the epiphyseal plate have been reviewed by Simpson Asling and Evans [23] A more extensive discussion of the effects of dietary factors on bone growth may be found in the monograph of Weinmann and Sicher[24] and the review of Silberberg and Silberberg [5]

Antibiosis Since the hypophysectomized animal is a valuable one and yet is easily subject to infection the effect on the growth assay of prophylactic

Choice of Animals In presenting the criteria whereby the hypophysectomized rat qualifies for the weight test Marx, Simpson and Evans [1] emphasized *that animals whose body weight gain exceeded 7 grams in the* pre injection period should be eliminated This criterion is of sufficient importance for all assays of growth hormone and has been justly re emphasized by Greenspan, Li Simpson and Evans Some limitation with respect to weight loss is equally justifiable experience dictating the permissible maximum to be three grams

The sex of the test animal used in previous studies had always been female However experiments conducted in this laboratory have established unquestionably that the male rat is perfectly comparable to the female with respect to sensitivity to growth hormone maximal response which they are capable of achieving and slope of the dose response curves furthermore, the standard errors of the responses are similar Consequently male *rats have been employed by us and with perfectly satisfactory results*

Postoperative Interval It may be recalled that rapid narrowing of the epiphyseal disc occurs within the first 10–12 days following hypophysectomy (Fig 1) thereafter the thickness of the disc remains essentially unchanged In order to take advantage of this period of maximal sensitivity in responsiveness the assay should begin within a short time following hypophysectomy but yet not before the control disc width has been sufficiently stabilized in order to avoid undue variation in response The suggested interval of 12 to 14 days fulfills these requirements

The 4 day Maintenance Test In some laboratories however a 4 day maintenance assay may be of more practical use than the present stimulation procedure The advantage of such a modification lies in the immediate use of the hypophysectomized animal eliminating problems of space feed and care and to some extent postoperative mortality

As noted earlier Becks Kibrick Marx and Evans[11] reported on the early effect of hypophysectomy and of immediate administration of growth hormone on the width and histology of the epiphyseal cartilage In these experiments a group of rats hypophysectomized at 26 days of age were injected for 4 days with 40 micrograms daily of a growth hormone preparation starting on the day of operation On day 30 they were sacrificed and compared with normal and hypophysectomized control animals It was found[11] that whereas the operated control animals showed a decrease of approximately 30 per cent in the width of their epiphyseal cartilages the animals maintained on growth hormone showed cartilage widths indistinguishable from the normal control group These results suggested that an assay with sensitivity *equal to that of the regular procedure might be* formulated in the form of a maintenance assay Consequently a number of experiments were instituted to determine the dose response characteristics of maintenance therapy Female rats were hypophysectomized at 27 days of age and, starting on the day of operation different groups of animals

dose of growth hormone divided into two equal injections (8 a m and 5 p m) in no way differs from that resulting from single daily injections

Length of the Injection Period In their original report on the tibia test assay Kibrick et al [14] reported that in the course of daily intraperitoneal injections of growth hormone a rapid increase in cartilage width was observed up to the fourth day with a leveling of the response beginning at the sixth day of injection Greenspan, et al [16] have also inquired into the effect of the period of injection finding that administration of the hormone for 1 to 2 days did not allow a maximal reaction while daily injections for either 4 or 5 days permitted a maximal response to be obtained The responses induced by growth hormone administered for either 3 or 4 days were found to be comparable up to a certain upper limit which for the 3-day period was 290 micra and for the 4 day period 340 micra This means as our own recent experience has indicated that the lower plateau attained by the response following the 3-day injection period offers no great practical drawback since the hormone is usually assayed at dose levels yielding responses that fall in the middle of the dose response curve For example in parallel experiments in which total doses of 20 60 and 120 micrograms of a growth hormone preparation were injected over a period of 3 or 4 days no significant difference was found in the responses at each dose level so that similar dose response curves could be constructed Furthermore the standard error of the response at each dose level in the 3-day test was not significantly different from that for the longer injection period

In summary either male or female hypophysectomized rats which in 12 to 14 days following hypophysectomy at 26 to 28 days of age show neither excessive weight gain nor loss have proven to be satisfactory assay animals Moreover such animals must be kept on an adequate diet and should not be subjected to antibiotic treatment in the period immediately preceding initiation of hormone administration Neither the mode of injection of the hormone the nature of the injection vehicle nor the frequency of injection produces any modification of the response to a given total dose of hormone Finally for all practical purposes the total dose of hormone may be injected over a period of 3 4 or 5 days with little or no difference in the responses

Specificity of the Tibia Test

Shortly after the introduction of the tibia test as an assay procedure for growth hormone Marx Simpson and Evans[17] reported on the specificity of the method They found that while the cartilage width was slightly decreased by the action of ACTH on the other hand a slight enlargement of the cartilage was produced by thyroxin and to a lesser extent by thyrotropic and lactogenic hormones Characteristic of this non specific increase in disc width was the failure of the cartilage to exceed a final width of 200

antibiotic therapy with either aureomycin or Terramycin® has been investigated In a typical experiment which we recently have performed 2 5 mg of Terramycin® was administered intraperitoneally every other day for a period of 16 days, starting on the day of operation At sacrifice these animals possessed discs with an average width of 176 micra compared to a control value of 163 micra In other experiments with the two antibiotics mentioned above values up to 196 micra have been obtained When injections of growth hormone are superimposed upon the administration of antibiotics the growth response is usually found to be enhanced probably as a result of a more favorable substrate for growth activity However, a great degree of variation in response is obtained since not all animals appear to react equally to the course of administration of antibiotics

Mode of Injection and the Nature of the Injection Vehicle The effect of varying the route of administration of the hormone has been previously investigated by Greenspan and his co workers [16] No significant differences in the mean responses were observed among groups of animals which received a standard dose of growth hormone either subcutaneously intraperitoneally or intravenously Our own results have been entirely confirmatory with respect to these findings

In addition to the experiments in which the mode of injection was varied we conducted others to determine the effect of changing the vehicle for injection The media employed were 25 per cent polyvinylpyrrolidone 5 per cent beeswax peanut oil 16 per cent gelatin 2 per cent polyphloretin phosphate (pH 5) and 2 per cent hesperidin phosphate (pH 8) The growth hormone has also been administered in the form of a suspension in zinc phosphate at pH 6 All solutions or suspensions were administered subcutaneously in volumes of 0 1 to 0 25 ml daily None of these delaying or protective vehicles was capable of enhancing the hormonal effect over that obtained with a simple aqueous solution injected subcutaneously As a matter of fact administration of the hormone in solutions of polyphloretin phosphate or hesperidin phosphate resulted in a definite inhibition of the response this result can be attributed to the toxicity of the additives since rather complete inhibition was also observed when the diluent and the growth hormone in simple aqueous solution were administered separately at different injection sites

Since some of the protective media exert their effect through an anti hyalouronidase action [1] the growth hormone was administered subcutaneously in a solution containing 250 turbidity reducing units of hyalouronidase (Wydase® Wyeth) per daily injection volume The hyalouronidase solution proved to be without effect on either the control level or on the response elicited by the hormone

Frequency of Injection We have confirmed and extended the findings of Greenspan et al [16] which indicate that the response obtained following the administration either subcutaneously or intraperitoneally of a daily

Table 3

THE SYNERGISTIC EFFECT OF THYROXIN (Tx) ON THE RESPONSE TO GROWTH HORMONE (GH)

Preparation	*Daily Dose** (µg)	*No of Rats*	*Width of Uncalcified Cartilage Plate (micra)*	*GH Equiv †* (µg)
Tx	0 25	9	171	~0
GH(L2189D)	6 25	7	208	17
Tx + GH	0 25 + 6 25	10	231	42
Uninjected Controls	—		165	0

* Administered for a period of 4 days
† From standard curve Fig 4

tion‡ increased the cartilage width to 192 micra The co administration of this dose of TSH with a standard dose of growth hormone did not produce *any widening of the cartilage greater than what would be produced by* growth hormone itself A significantly greater response could be obtained with growth hormone however following maintenance therapy with 200 micrograms daily of TSH It is evident that very large doses of thyrotropic hormone are required to duplicate the results obtained with microgram units of thyroxin The dosages of TSH required to produce an effect are so large that in the small amount of TSH expected to exist as a contaminant of growth hormone it would have little or no effect on the response

In further pursuit of the problem of the interaction of the thyroid with growth hormone a series of experiments was undertaken with doubly operated thyroidectomized hypophysectomized animals [8] It was observed that such test animals were unresponsive to injections of growth hormone the order of sensitivity being about one twentieth that of the hypophysectomized animal However if a maintenance dose of thyroxin is administered to these animals their sensitivity to growth hormone is partially restored

It seems apparent from all of these results that the tibia line test is a sensitive test because of a small amount of thyroxin still being secreted by the thyroid of the hypophysectomized animal Scow Simpson Asling Li and Evans [9] had arrived at a similar conclusion in explaining why growth hormone is less effective than thyroxin in stimulating growth of thyroidectomized animals

Adrenal Steroids ACTH and the Adrenal The observation that ACTH administration leads to further narrowing of the epiphyseal cartilage of hypophysectomized animals was first reported by Marx Simpson Li and Evans [30] When ACTH and growth hormone were both *given to test animals*

‡ Obtained through the courtesy of Dr S Steelman of the Armour Laboratories

micra as well as the lack of proportionality between dose and response The details of these and other more recent experiments are reported below

Thyroxin, Thyrotropic Hormone and the Thyroid * The effect of thyroidectomy in producing almost complete retardation of skeletal maturation is now well recognized as is the synergistic effect of thyroxin on osseous growth induced by growth hormone (see ref 23 for a detailed review) There seems little question then that the trinity of thyrotropic hormone thyroid gland and thyroxin exerting their effects through the agency of thyroxin (or triiodothyronine) may influence the width of the epiphyseal cartilage by themselves or may modify the response to growth hormone It has been pointed out above that Marx *et al* found that thyroxin produced a non specific increase in the width of the cartilage These increases were found following administration of from 5 to 30 micrograms of thyroxin (presumably DL) daily We have confirmed these results finding an average width of 201 micra in 26 rats injected with 2 micrograms daily of L thyroxin compared with a control value of 165 micra By administering thyroxin jointly with growth hormone during the 4 days of the injection period a new standard dose response curve can be constructed which has essentially the same slope as the curve in Figure 4 However, the whole curve is displaced toward the left in the direction of lower total doses of growth hormone This curve indicates that the synergistic effect of thyroxin with growth hormone effectively increases the response to equal that obtained from triple the amount of growth hormone alone A similar curve may be constructed after the injection of graded doses of growth hormone into rats which have been maintained during the entire postoperative period with 2 micrograms daily of L thyroxin Control animals maintained in this manner possess an average cartilage width of 218 micra Similar results for both types of experiment have been obtained with daily doses of thyroxin ranging from 0 5 to 10 micrograms In Table 3 the results are presented of experiments in which a standard dose of growth hormone was administered together with only 0 25 microgram daily of L thyroxin Since at this dose level thyroxin alone produces little or no increase in cartilage width the synergistic effect of thyroxin is more obvious

The import of these experiments insofar as they concern the assay of pituitary preparations for growth hormone activity lies in the effect produced by contamination with thyrotropic hormone When we administered 200 micrograms per day of a thyrotropic hormone preparation † for a period of 4 days the resulting epiphyseal cartilage width did not differ from the control value *A daily dose of 500 micrograms of another TSH prepara*

---

* Much of the data in this section has served as the basis of two previous reports[27, 28] from this laboratory

† Obtained through the courtesy of Dr I Gordon Fels of the Institute of Experimental Biology Berkeley

Table 4

GROWTH HORMONE ACTH ANTAGONISM *

Group	Total Dose (μg)	Width of Uncalcified Cartilage Plate (micra)	Two Adrenals Wt (mg)	Thymus Wt (mg)
Control	—	163	7.9	164
GH(L249B)	60	244	8.3	176
α Corticotropin†	100‡	148	11.8	87
GH + α-corticotropin†	60 + 100	251	12.4	109
α Corticotropin§	100‡	116	19.3	44
GH + α-corticotropin§	60 + 100	126	20.9	44

* 10 animals per group
† Administered intraperitoneally in aqueous solution
‡ Equivalent to 10 USP units
§ Administered subcutaneously in peanut oil beeswax suspension

mortality[3] of the operated animals. Preliminary experiments revealed that 1 mg daily of DOC administered for 4 days had little effect in the hypophysectomized animal either on the control response or on the response to growth hormone. Seven days of DOC administration produced a slight decrease in both the control and growth hormone response values. After 10 days of injection however a marked decrease was found in the response to growth hormone. It was decided therefore to adrenalectomize the animals 10 days after hypophysectomy that is 3 days prior to the start of the growth hormone injection period. This would allow the animals sufficient time to overcome the effects of operative trauma and yet minimize the period of time over which DOC would have to be administered. With this regime all animals appeared to be in good health and no mortality was encountered in the 8 days following adrenalectomy. The increase in body weight of all singly operated animals who received DOC was of the order of 1 gram per day. The results of these investigations (Table 5) demonstrated that the average disc width of the doubly operated group receiving DOC alone was significantly smaller than that of the comparable singly operated groups this was reflected in turn in the difference between the two groups receiving injection of both DOC and growth hormone. It should be noted however that the mean increase produced by growth hormone in all groups was approximately 80 micra. Thus it would appear that the doubly operated animal receiving DOC responds as sensitively to 4 days of growth hormone therapy as does the usual assay animal. These results complement the reports of Levie and Uyldert[33] and of Simpson Marx Becks and Evans[32] who have made similar findings in salt and glucose maintained adrenalectomized and hypophysectomized adrenalectomized animals injected with growth hormone for longer periods of time. Such

values intermediate between those given by ACTH or growth hormone alone were obtained These data were obtained from animals which had been injected daily for 2 weeks with the hormones in question they were extended to the 4 day injection period of the tibia test by Marx et al[17] who showed that daily doses of 25 to 500 micrograms of an ACTH preparation (approximately 1 USP Unit per mg) were capable of producing slightly narrower cartilage discs than those of the controls Subsequent experiments have shown that the degree of antagonism between growth hormone and ACTH depends to a large extent, upon the ratio of the two hormones Furthermore a wide variation has been encountered in the ability of various crude ACTH preparations to antagonize growth hormone We reported* at the time (1952) that ACTH preparations with good adrenal weight maintenance activity showed more marked antagonism than those with poor activity—quite independent of the unitage of the preparations as determined by the intravenous adrenal ascorbic acid procedure of Sayers et al[19] We have very recently reinvestigated the problem of antagonism employing a highly purified preparation of α corticotropin[31] which in some experiments has been admixed with growth hormone in aqueous solution and then administered intraperitoneally and in others has been injected subcutaneously in beeswax peanut oil suspension These experiments have demonstrated (Table 4) that in aqueous solution extremely high doses of α corticotropin have little effect on the tibial cartilage width either when acting alone or in conjunction with growth hormone despite the fact that significant involution of the thymus has occurred When administered in a delaying medium beeswax peanut oil α corticotropin causes a very marked thymic involution a highly significant decrease in the control cartilage width and an almost complete inhibition of the response to growth hormone Insofar as the practical aspects of the assay are concerned gross contamination of a growth hormone preparation with ACTH should not significantly alter the expected response to the growth hormone when the injections are given as they usually are i e intraperitoneally in an aqueous medium

The earlier work with ACTH is considered to have answered the problem concerning the role of the adrenal gland on bone growth and few extensive studies relating to this subject have been published in which synthetic adrenocortical steroids were employed We have had occasion to study the effects of hydrocortisone on the cartilage widths of normal animals these have been reported earlier In the course of an earlier investigation on the use for the assay of growth hormone preparations contaminated with ACTH of the doubly operated hypophysectomized adrenalectomized animal we undertook a study on the effects of 11 desoxycorticosterone (DOC) with the expectation of using this steroid to decrease the high

* Reported at the Annual Meetings of the Endocrine Society June 1952 See ref 27

discussions in refs 34 and 35) We have repeated those experiments which have some bearing on the significance of the tibia test and the results are recorded in Table 6 These data demonstrate a highly significant ($P < 0.001$) augmentation of the growth hormone response by 1 mg daily of testosterone the steroid alone does not alter the control cartilage width However no significant augmentation was evident when doses of growth hormone were employed which themselves gave responses of the order of 250 micra or more (comparable to the conditions employed by Simpson et al [31]) The nature of a logarithmic function curve is such that with a reasonable variation in the response a small augmentation would not be readily detectable at very high levels of response When 0 1 mg daily of testosterone was employed rather than 1 mg a small but significant increase in the control cartilage width was observed as well as an even greater enhancement of the tibial response to growth hormone than that reported above and in Table 6 Thus a daily dose of 0 005 mg of a growth hormone preparation (L2518B) resulted in an average cartilage width of 196 micra whereas the hormone given together with 0 1 mg daily of testosterone produced an average width of 231 micra

Table 6

THE EFFECT OF ADMINISTRATION OF TESTOSTERONE PROPIONATE (TP) OR ESTRADIOL BENZOATE (EB) ON THE RESPONSE OF THE TIBIAL CARTILAGE TO GROWTH HORMONE (GH)

Series	*Experimental Condition*	*Daily Dose (mg)*	*No of Animals*	*Width of Tibial Epiphyseal Cartilage Plate (micra)*	*GH Equiv †(micrograms)*
I	TP	1 0	18	165 (159–176)‡	0
	GH	0 0075	14	221 ± 2 0§ (207–230)	28
	TP + GH	1 0+ 0 0075	14	237 ± 1 9 (225–249)	54
II	EB	0 5	5	158 (150–164)	0
	GH	0 015	10	236 ± 2 6 (222–246)	52
	EB + GH	0 5+ 0 015	9	219 ± 3 4 (200–231)	26

Testosterone and estradiol administered subcutaneously in a volume of 0 1 ml
† From standard curve Fig 4
‡ Range of responses in parentheses
§ Mean ± standard error

In a companion study to one concerned with the effect of estrin implants on the tibia of young normal female rats Kibrick Simpson Becks and Evans[36] reported on the lack of effect of estrin implants on the tibias of hypophysectomized rats We have injected animals for 4 days with 0 5 mg daily of estradiol benzoate and have found that although administration of

Table 5

THE EFFECTS OF DESOXYCORTICOSTERONE (DOC) AND OF GROWTH HORMONE
*IN THE HYPOPHYSECTOMIZED-ADRENALECTOMIZED RAT**

Operative Condition†	*Total Dose*		*Width of Uncalcified Cartilage Plate*
	DOC‡ (*mg*)	*GH* (μ*g*)	(*micra*)
$\overline{H}$	0	0	165
$\overline{H}$	7	0	149 (142–159)§
$\overline{H}$	0	40	234 (229–241)
$\overline{H}$	7	40	230 (222–238)
$\overline{H}$-$\overline{A}$	7	0	121 (104–142)
$\overline{H}$-$\overline{A}$	7	40	201 (188–220)

* Hypophysectomy ($\overline{H}$) at day 27 Adrenalectomy ($\overline{A}$) at day 37
† $\overline{H}$—6 animals per group $\overline{H}$-$\overline{A}$—8 animals per group
‡ Administered daily for 7 days beginning on day 37
§ Range of responses in parentheses

doubly operated animals receiving DOC are useful for assaying the degree to which an ACTH preparation is contaminated by growth hormone

Gonadal Steroids, Gonadotropins and the Gonads Few reports exist of the effects of gonadotropins per se on skeletal growth This problem has been attacked mainly through study of the effects of ablation of the target organs or by consideration of the changes produced by administration of *the gonadal steroids Among the steroids* the greatest attention has been paid to the effects of testosterone and the estrogens whereas little has been reported with respect to the effects of progesterone administration on bone growth

The results of administration of testosterone to hypoyhysectomized animals have been reported by Simpson Marx Becks and Evans[34] and by Reiss Fernandes and Golla[3] The former authors reported an increase in the width of the epiphyseal cartilage following treatment long after operation of hypophysectomized female rats with 0 1 or 1 mg daily of testosterone for 10 days However under the conditions of the growth hormone assay procedure daily injections of 0 05 or 1 mg of the steroid produced no detectable change from the control group The injection of 0 25 mg daily led to some augmentation (probably not significant) of the response to a daily dose of 100 micrograms of growth hormone Reiss et al[3] however have claimed that daily injection of 4 mg of testosterone for 14 days markedly inhibited cartilaginous growth produced by 5 mg daily of a growth hormone preparation This lack of agreement between these two groups of workers is magnified many fold in the literature on the effects of castration or of testosterone administration in experimental animals (see

Table 7

THE EFFECT OF LACTOGENIC HORMONE ON THE WIDTH OF THE PROXIMAL EPIPHYSEAL CARTILAGE OF THE TIBIA

Preparation	*Daily Dose (mg)*	*No of Rats*	*Sex of Rats**	*Width of Uncalcified Cartilage Plate (micra)*
2C113G	2 5	9	F	222 (203–247)†
	2 5	9	M	260 (242–278)
4C15	2 5	5	F	197 (190–207)
	2 5	5	M	231 (227–238)
B I	0 25	5	M	213 (201–223)
	0 5	5	M	214 (210–218)
	0 5	5	F	178 (172–185)
	0 625	5	M	225 (223–227)
	1 25	5	M	220 (214–231)
	2 5	9	M	226 (218–243)
B I boiled	0 625	5	M	211 (203–220)
	2 5	5	M	223 (211–233)
B III‡	0 5	5	M	229 (218–240)
	0 5	5	F	183 (178–185)
3C50B	0 5	5	M	182 (177–191)
3C82B	0 5	5	M	178 (172–185)

* M = male F = female
† Range of responses in parentheses
‡ Assay animals were doubly operated castrated at 26 days of age and hypophysectomized at 28 days of age

activity of lactogenic hormone [39] no marked loss of activity with respect to the tibia test was observed (Table 7) However when growth hormone (40 micrograms per ml) was boiled under these same conditions in the presence of casein (5 mg per ml) complete loss of growth activity was observed Other experiments demonstrated that lactogenic hormone acted neither as an inhibitor nor as a synergist to increase the response to growth hormone in male and female rats rather the response obtained was a simple summation of effects (Table 8) Still other experiments demonstrated that at least in the female testosterone propionate acted as a synergist to increase the response to lactogenic hormone (Table 8)

The possibility existed that sex dependence was a characteristic of the non specific response however neither the response to thyroxin nor to progesterone showed this dependence In the hope that use of the doubly

the steroid does not affect the control level it significantly (P = 0 001) depresses the response to a standard dose of growth hormone (Table 6) Progesterone administration (1 mg daily) was found to promote a slight increase in the cartilage width of the control groups and to be without effect on the response to growth hormone Thus among the gonadal steroids testosterone enhances the response to growth hormone estradiol depresses it and progesterone has no effect Since rather large doses of an estrogen are required to produce an inhibition of the growth hormone response (smaller doses are without effect) any contamination of growth hormone preparations with the gonadotropins responsible for estrogen production would not be expected to alter the response to growth hormone If the male assay animal is employed however contamination with ICSH may produce a significantly increased response to growth hormone for only small quantities of testosterone are required to enhance the effect Whether small quantities of ICSH could be expected, 13 days after hypophysectomy to cause sufficient production of testosterone (or other testicular steroids) during a period of 4 days is not known

Lactogenic Hormone * The one gonadotropin about whose actions on the skeleton some knowledge exists is lactogenic hormone Marx et al [17] reported that daily doses of 0 5 to 4 mg of a lactogenic hormone preparation caused only a slight enlargement of the epiphyseal cartilage Simpson et al [3] have cited the results of some experiments in which hypophysectomized rats were injected with lactogenic hormone but only scant information has thus far appeared

In the course of determining the degree of growth hormone contamination in some lactogenic hormone preparations being prepared in this laboratory some very striking results were obtained which inspired a more complete investigation of the effects of this hormone on the tibia test At the same time that these first preparations were to be assayed the investigation comparing the usefulness of the male and female hypophysectomized rat was being undertaken hence one of the lactogenic hormone preparations was assayed in both male and female rats This preparation (2C113G) prepared essentially according to the method described by Li Simpson and Evans [37] showed definite evidence of growth hormone contamination and in addition what is more interesting showed a sex dependent response (Table 7) With preparations (4C15 and B I) which had been submitted to counter-current distribution [35] the degree of the response was smaller but the sex difference remained It was further observed that the response to lactogenic hormone in the male reached a maximum of approximately 225 micra When a preparation in 0 5% neutral solution was boiled for 15 minutes conditions which are known to have little effect on the crop sac

* The experiments on lactogenic hormone were carried out in collaboration with Dr R D Cole of this laboratory

operated hypophysectomized-castrated rat would supply some information concerning the basis of this sex dependence rats of both sexes were castrated at 26 days of age and hypophysectomized 2 days later Administration of lactogenic hormone (B III) to these animals produced the typical sex dependent response (Table 7)

It will be recalled that the first lactogenic hormone preparation employed 2C113G evoked a marked response even in female assay animals This was in contrast to the findings of Marx et al [17] who as noted above found only slight enlargement of the cartilage with comparable doses of the hormone The hormone employed by these workers was prepared by the method of Lyons[40] which involves treatment of the preparations with ammonium hydroxide solutions of one third concentration for 3 hours at room temperature The method of Li et al [37] used for the preparation of 2C113G omits this step In order to test the possibility that treatment of the hormone with ammonia in some way destroys that activity which is measured by the tibia test two preparations of the hormone prepared by the method of Li et al were dissolved in ammonium hydroxide solution From these solutions the hormone was re isolated (3C50B and 3C82B) and then tested for growth activity The results of these assays demonstrate an almost complete loss of such activity in the male rat following treatment with ammonia (Table 7) These preparations are fully active in the crop sac assay and the only change in their chemistry that has been found is an alteration of their electrokinetic behavior (personal communication from Dr R D Cole)

There seems little question that preparation 2C113G was contaminated with growth hormone to the extent of 0 3 per cent Purification of such a preparation by means of counter current distribution eliminates the bulk of the growth hormone contaminant but apparently leaves behind another factor which shows activity according to the tibia test This factor however differs in several respects from growth hormone (1) the response elicited by it is dependent upon the sex of the assay animal (2) it is more resistant to inactivation by boiling and (3) the maximal increase in tibial width that it induces is approximately 225 micra as compared with approximately 330 micra produced by growth hormone These differences cannot be explained in terms of any possible modification of the effect of the contaminant by the lactogenic hormone itself since previous experiments which were carried out on the effect of growth hormone added to lactogenic hormone demonstrated that the response of the tibia to the former is unaffected by the latter in female rats and is not synergistically enhanced in male this would leave the sex dependent response still unexplained The same experiments also indicate that the lactogenic hormone does not interfere with the maximal tibial response of 330 micra produced by growth hormone For these reasons the existence of a discrete factor is suggested In terms of the tibia test such a factor—if it exists would be

Table 8

THE EFFECTS OF ADMINISTRATION OF LACTOGENIC HORMONE (PROLACTIN) TOGETHER WITH GROWTH HORMONE (GH) OR TESTOSTERONE PROPIONATE (TP) ON THE "TIBIA TEST"

Series	*Experiment*	*Daily Dose** *(mg)*	*Sex of Rats*	*Width of Uncalcified Cartilage Plate (micra)*	*GH Equivalent†* *(micrograms)*	*Theoretical GH Equivalent‡* *(micrograms)*
I	Prolactin	0 25	F	165 (161–170)§	0	
	GH	0 015	F	236 (216–252)	53	
	Prolactin + GH	0 25+ 0 015	F	235 (227–243)	51	53
II	Prolactin	0 25	M	220 (209–232)	27	
	GH	0 015	M	237 (229–245)	55	
	Prolactin + GH	0 25+ 0 015	M	245 (236–258)	75	82
III	Prolactin	0 5	F	178 (172–185)	~0	
	TP¶	1 0	F	165 (159–176)	0	
	Prolactin + TP**	0 5+ 1 0	F	203 (192–215)	14	~0

* All experimental groups contain 5 animals unless otherwise noted
† From standard curve Fig 4
‡ Based on simple summation of equivalents
§ Range of responses in parentheses
¶ 18 animals per group
** 10 animals per group

Conclusions

Measurement of the increase in the width of the proximal epiphyseal cartilage of the tibia is the most sensitive test known for the determination of growth hormone activity Additional advantages derive from the small amount of hormone required to perform the test and the short duration of the assay procedure compared with the other recognized tests for growth hormone activity As has been shown in this paper when the assay is performed in accordance with recognized statistical design and with a standard growth hormone preparation excellent reproducibility of assay results for an unknown preparation may be expected

For growth hormone preparations the test is also very specific The expected contaminants of such preparations namely the other pituitary hormones in the range they would be encountered in such preparations would not be expected to affect the response of the hypophysectomized female rat to growth hormone the male rat *may* respond differently However the standard assay procedure is of little use in determining the amount of a growth hormone contaminant in preparations of other pituitary hormones for with hormonal doses of the magnitude that would ordinarily be employed to reveal a growth hormone contaminant the major hormone would probably affect either the control disc width or the response to the contaminant For such occasions the removal of the target organ for the major hormone in addition to removal of the hypophysis results in an apparently adequate assay animal In the specific case of the thyroidectomized hypophysectomized rat replacement therapy with 0 5 to 2 micrograms daily of thyroxin is necessary to restore partially the sensitivity of the end organ s response to growth hormone In the case of the adrenalectomized hypophysectomized animal maintenance therapy with desoxycorticosterone has been employed to decrease the mortality rate

Experiments with lactogenic hormone preparations have suggested the existence of a growth factor which causes an increment in the width of the tibia but which has characteristics entirely different from growth hormone itself for example it invokes a sex dependent response it is more resistant to inactivation by boiling and the maximal response which it induces is lower than that produced by growth hormone Further studies are necessary to clarify the nature of this factor

References

1 Dott N M and J Fraser *Quart J Exp Physiol Suppl* 13 107 (1923)
2 Dott N M *Quart J Exp Physiol* 13 241 (1923)
3 Handelsman M B and E F Gordon *J Pharmacol* 38 349 (1930)
4 Lucke H and R Huckel *Arch exp Pathol Pharmakol* 169 290 (1933)
5 Silberberg M *Proc Soc Exp Biol Med* 32 1423 (1935)
6 Freud J Levie L H and D B Kroon *J Endocrinology* 1 56 (1939)
7 Ross E S and F C McLean *Endocrinology* 27 329 (1940)

expected to have little effect in female assay animals but conceivably could *affect the response to growth hormone of male assay animals*

Other Endocrine Factors (1) *Hormones of the Pars Nervosa and Pars Intermedia* Nothing is known of the effect on the tibia test of these hormones ubiquitous contaminants of anterior pituitary preparations It would be expected that the toxicity of Pitressin® so very marked in hypophysectomized rats would depress the response to growth hormone

(2) *Hormones of the Parathyroid Glands* Parathyroid extracts and parathyroid gland ablation have very well known effects on skeletal growth and development Experiments carried out in this laboratory with parathyroid extracts (Lilly) have shown that the hypophysectomized rat can tolerate only about one twentieth of the unitage of such commercial extracts as the normal animal However daily doses of from 5 to 10 units do not affect the response to a standard dose of growth hormone

(3) *Hormones of the Pancreas* Both pancreatic glucagon and insulin have been implicated in the physiological responses to growth hormone Elrick[41] reported that glucagon produced a slight increase in the tibial cartilage disc width but Geschwind and Staub[4] were unable to confirm this with a highly purified glucagon preparation

We have left for last a discussion of the action of insulin on the width of the cartilage plate In a highly interesting report Salter and Best[43] have suggested that insulin can function as *a* growth hormone and have reported a widening (increment of approximately 75 micra) of the tibial epiphyseal disc following administration to hypophysectomized rats of gradually increasing doses of protamine zinc insulin for 15 days beginning 2 weeks postoperatively These increments however were well below those produced by growth hormone administration to such animals We have endeavored to determine the effects of zinc insulin or protamine zinc insulin (Lilly) administered during a 4 day period on the tibia test Zinc insulin has been injected intraperitoneally in a daily dose of 0 02 unit with no mortality resulting among the assay animals if 1 ml of a 20 per cent glucose solution was injected intraperitoneally one half hour after the hormone injection With this dose of insulin no effect could be observed on the cartilage width More recently we have administered subcutaneously 0 1 unit daily of protamine zinc insulin Even with constant care and repeated glucose injections a 70% mortality was encountered In those animals which did survive a definite depression of the control cartilage disc width was observed Our conditions differ from those described by Salter and Best in that our standard assay animal is hypophysectomized at an earlier age that the animals employed were female and undoubtedly of greatest importance that we did not use the same diet To alter the assay procedure to conform with these differences would result in a modified procedure yielding results no longer comparable with those obtained from the standard procedure and no longer relevant to this discussion

37 Li C H Simpson M E and H M Evans *J Biol Chem* 146 627 (1942)
38 Cole R D and C H Li *Fed Proc* 13 193 (1954)
39 Riddle O and R W Bates *Sex and Internal Secretions* Ed E Allen Baltimore The Williams & Wilkins Co 1939
40 Lyons W R *Cold Spring Harbor Symposia Quant Biol* 5 198 (1937)
41 Elrick H *Proc Soc Exp Biol Med* 81 15 (1952)
42 Geschwind I I and A Staub *Proc Soc Exp Biol Med* 84 244 (1953)
43 Salter G and C H Best *Brit Med J* 2 353 (1953)

DISCUSSION

Bioassay, Preparation and Physicochemical Properties of Growth Hormone

Designated Discussion

ALBERT SEGALOFF (Alton Ochsner Medical Foundation) We have seen this morning some truly excellent results of a great deal of study Dr Russell is to be complimented not only for working out what appears to be a reasonably short assay for growth hormone effect on nitrogen metabolism but for achieving an assay which is quite reproducible Those of us who have attempted unsuccessfully to do this realize how much work Dr Russell s study represents

The tibial line assay is one of great sensitivity and we agree thoroughly with Dr Geschwind in respect to the factors which are involved in the assay There is one additional point which I think should be mentioned and this relates to the application of the tibial line assay and other assays to biological fluids either from man or animal We have been particularly interested in the growth hormone assay of plasma and mentioning this brings up an additional thought in regard not only to the circulating hormones but to the plasma proteins and other constituents of plasma Thus I might add to Dr Geschwind s discussion that we have been unable to demonstrate any significant effect on the assay of growth hormone preparations when plasma proteins are added in amounts which we have used in assaying the growth hormone content of plasma As far as the assay effects of thyroxin and TSH are concerned it has been our finding that thyroxin in amounts comparable to that contained in plasma used in the four day assay has no significant influence on the sensitivity of growth hormone We feel therefore that in assaying plasma circulating thyroxin is not a factor The same is true for ACTH At the moment I don t know how to interpret the effect of the prolactin We too have seen some effect from prolactin preparations which we had interpreted as being due to growth hormone contamination I am afraid we must now take another look at the problem of prolactin to determine whether or not the amount in plasma produces a significant contribution

8 Ingalls T H *Endocrinology* **29** 710 (1941)
9 Ingalls T H and D R Hayes *Endocrinology* **29** 720 (1941)
10 Ray R D Evans H M and H Becks *Am J Path* **17** 509 (1941)
11 Becks H Kibrick E A Marx W and H M Evans *Growth* **5** 449 (1941)
12 Becks H Simpson M E and H M Evans *Anat Rec* **92** 109 (1945)
13 Becks H Simpson M E and H M Evans *Anat Rec* **92** 121 (1945)
13a Gaarenstroom I H and L H Levie *J Endocrinology* **1** 420 (1939)
13b Becks H Simpson M E Li C H and H M Evans *Endocrinology* **34** 305 (1944)
14 Kibrick E A Becks H Marx W and H M Evans *Growth* **5** 437 (1941)
15 Evans H M Simpson M E Marx W and E Kibrick *Endocrinology* **32** 13 (1943)
16 Greenspan F S Li C H Simpson M E and H M Evans *Endocrinology* **45** 455 (1949)
17 Marx W Simpson M E and H M Evans *Proc Soc Exp Biol Med* **55** 250 (1944)
18 Li C H Evans H M and M E Simpson *J Biol Chem* **159** 353 (1945)
19 Sayers M A Sayers G and L A Woodbury *Endocrinology* **42** 379 (1948)
20 Li C H Clauser H Fonss Bech P Levy A L Condliffe P and H Papkoff see elsewhere in this volume
21 Marx W Simpson M E and H M Evans *Endocrinology* **30** 1 (1942)
22 Greenspan F S Li C H Simpson M E and H M Evans *Hormone Assay* Ed C W Emmens New York Academic Press Inc 1950
23 Simpson M E Asling C W and H M Evans *Yale J Biol Med* **23** 1 (1950)
24 Weinmann J P and H Sicher *Bone and Bones* St Louis The C V Mosby Co 1947
25 Silberberg M and R Silberberg *Growth* **13** 359 (1949)
26 Hamburger C *Acta Endocrinol* **11** 282 (1952)
27 Geschwind I I and C H Li *J Clin Endocrinol* **12** 937 (1952)
28 Geschwind I I and Li C H cited by C H Li *Bioassay of Anterior Pituitary and Adrenocortical Hormones* Ed G E W Wolstenholme London J and A Churchill 1953
29 Scow R D Simpson M E Asling C W Li C H and H M Evans *Anat Rec* **104** 445 (1949)
30 Marx W Simpson M E Li C H and H M Evans *Endocrinology* **33** 102 (1943)
31 Li C H Geschwind I I Levy A L Harris J I Dixon J S Pon N G and J O Porath *Nature* **173** 251 (1954)
32 Simpson M E Marx W Becks H and H M Evans *Endocrinology* **35** 234 (1944)
33 Levie L H and I E Uyldert *Acta Brevia Neerl* **9** 121 (1939)
34 Simpson M E Marx W Becks H and H M Evans *Endocrinology* **35** 309 (1944)
35 Reiss M Fernandes J E and Y M L Golla *Endocrinology* **38** 65 (1946)
36 Kibrick E A Simpson M E Becks H and H M Evans *Endocrinology* **31** 93 (1942)

reference standard The plasma level appears to go neither up nor down with the advancing years of our patients We have some additional data and the values all seem to stay within the same range

In summary allowing us the fact that the assay is specific and that the factor we are talking about is growth hormone it does exist in plasma at least and it does assay quite reproducibly

General Discussion

FRANCIS D W LUKENS (University of Pennsylvania) I would like to ask Dr Russell if she has used tri iodothyronine in an attempt to determine the relation of the thyroid to her rather short lasting test I would like to ask Dr Geschwind what is known about the tibia test in species other than the rat

JANE RUSSELL There is a very simple answer to that question No we have not had any tri iodothyronine to carry out the experiment

IRVING GESCHWIND The mouse is the only other species which I know has been used for the assay Miss Lastrow in our laboratory has used the hypophysectomized mouse with results which are comparable to those obtained in the hypophysectomized rat

ALBERT SEGALOFF We have also used the mouse with excellent results The immature mouse in our hands is about 10 times as sensitive as the imma ture rat It is interesting that in the immature guinea pig experiments we have found the same failure of growth of the tibial line as has been reported by the Montreal group In other words we have been unable to increase the tibial line width of the immature hypophysectomized guinea pig with beef or pork growth hormone preparations

HANS SELYE (University of Montreal) We have performed some experi ments which rather strikingly support what Dr Weiss said this morning and also what has been reported in connection with the tibia test namely that the same hormone can stimulate growth in one place and inhibit growth in another It was particularly unexpected to find that even in bone itself stimulation and inhibition may go parallel The data in Figure 2 illustrate this This experiment was published last year in *Annales d en docrinologie* but perhaps many of you haven t seen it that is why I brought the picture along On the right is the lower end of the femur of an animal treated with growth hormone or somatotropin (STH) We prefer to call it the latter because it doesn t mean so much On the far left is the femur of an animal which has been treated exclusively with an estrogenic preparation stilbesterol Naturally occurring estrogens work the same way It is evident that the estrogen alone produces some bone spicule deposition

There is one problem however which I don t know how to answer It relates to assay effects of antibiotics which Dr Geschwind discussed Considering the fact that many of our feeds now contain not inconsequential amounts of antibiotics may explain some of the week to week variations most of us find in the assays

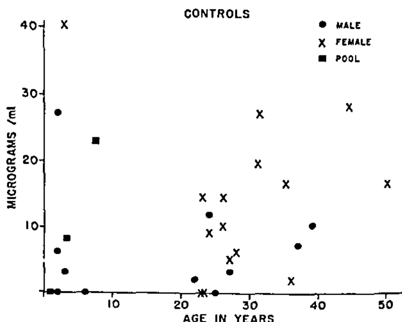


FIG 1

With these remarks I think it is interesting that in a large number of patients with clinical conditions and whose plasma samples we have attempted to assay for growth hormone we have not found a very great range In other patients particularly in the few with active acromegaly we have found plasma assay values which I think are well above anything due to chance In our patients with unquestionable pan hypopituitarism we have consistently found negative values In this group of patients we have seen a few who had not only measurable amounts of what we like to consider as growth hormone or at least tibial width increasing material but also detectable amounts of gonadotropins both in plasma and in urine These cases must be considered as clinical examples of incomplete pan hypopituitarism as far as assays go Now in Figure 1 I have plotted a few of our control values In essence we have found it necessary to use pooled specimens to obtain levels in most of the small children There are a few zero values which I am unable to explain This may not be necessary but there are some The highest value we have seen in these controls was 40 micrograms per ml equivalent to Armour s standard 22KR–2 which was our only

GEORGE MICHAELS (Highland Alameda County Hospital) I wish to present a procedure for the assay of pituitary growth hormone This is done by preparing cotton gauze squares of twelve fold thickness and of medium weave cut approximately 1 cm in diameter These squares are cut carefully so that the variation in weight between squares is within one milligram The experimental animal is the intact female rat weighing from 150 to 200 g

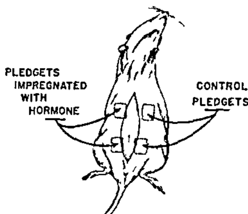


FIG 3 In vivo tissue culture technique for growth hormone assay

A median skin incision on the abdominal wall is made as shown in Figure 3 and two pledgets impregnated with the hormone are placed on one side of the rat directly under the skin two other untreated pledgets are placed on the opposite side and the incision is closed A second animal is handled in the same manner and four untreated gauze pledgets are inserted under the skin in the same fashion This represents the true control The entire procedure must be carried out under strictly aseptic conditions After nine days the rats are sacrificed and the pledgets are carefully dissected free, weighed and used for chemical analysis

Gauze pledgets which had been impregnated with 1 mg of Armour s pituitary growth hormone were implanted in 20 rats Twenty more rats were used as controls In Figure 4 are shown the mean nitrogen potassium and sodium values per gram of tissue which were found in this experiment There was significantly more nitrogen per gram of tissue in the growth hormone impregnated pledgets This amounted to 3 03 ± 0 40 mg or more than 7 times the standard error A smaller but significant difference was found between the true controls and the treated controls the difference of the means being 1 37 ± 0 4 milligram The potassium content of the treated pledgets was 0 28 mg ± 0 06 greater than that of the absolute con trols The sodium content per gram was 0 24 mg ± 0 06 *less than* that of the controls

but not very much Growth hormone alone increases the growth of the whole bone but does not have any selective sclerosing effect The two hormones together produce an inhibition of the tibial cartilage, which you can see in the center preparation At the same time however there is a tremendous synergism in respect to bone proliferation One might wonder whether some sort of synergistic treatment such as this could be developed in order to stimulate bone growth in problems of clinical medicine What ever the applications may be it is noteworthy that while inhibiting effects occur at the tibial junction line a synergism takes place elsewhere



FIG 2

RITA CARRIERE (McGill University School of Medicine) I would like to ask Dr Geschwind if he has tried using hypophysectomized thyroidec tomized animals for periods of say 3 weeks or so? If so did he get a con tinued response to growth hormone? Is the response enhanced by thyroxin and if so by how much?

IRVING GESCHWIND I am afraid I didn t make that point clear during my talk We have used hypophysectomized thyroidectomized animals and they showed a markedly depressed sensitivity to growth hormone If you ad minister small amounts of thyroxin to such animals you only partially re store their sensitivity to growth hormone The order of sensitivity in a doubly operated animal is only about 1/20th that of the hypophysectomized animal In respect to the duration of response to growth hormone I was concerned solely with that length of time involved in the assay period itself which is four days of injection The animals were thyroidectomized and hypophy sectomized two to three weeks previously

4

Comparative Biochemistry of Growth Hormone From Ox, Sheep, Pig, Horse and Fish Pituitaries

Alfred E Wilhelmi
Division of Basic Sciences in the Health Services Emory University Georgia

A growth hormone preparation from hypophyseal tissue and of high purity was first made by Li Evans and Simpson[1] in 1945 and in the years immediately preceding An improved method yielding much larger quantities of a crystalline product of a similar order of purity was described in 1948 by Wilhelmi Fishman and Russell[2] Ox pituitaries were the source material for both of these preparations which have been studied most extensively and used widely in work on the biological activity of growth hormone Raben and Westermeyer[3] in 1951 described the preparation of an active growth hormone as a by product of the preparation of ACTH from pig pituitaries This material prepared by a method radically different from those used for the isolation of the hormone from ox glands is also strikingly different in its solubility properties No detailed chemical study of the growth hormone prepared from pig pituitaries seems to have been published Thus far then the only preparations of purified growth hormone which have been made available for physical chemical and biological study are those derived from ox and pig pituitaries

During the past five years work in our laboratory has furnished opportunity for the isolation and study of active growth hormone preparations from ox sheep pig horse and fish pituitary glands The observations are at present somewhat unsystematic and by no means complete This is due in part to the scarcity of some of the starting materials and to the urgent demands upon the product for other work In part it is due to limitations of time which have not allowed us to do all of the things that clearly should be done This paper is therefore only a preliminary report on some comparisons between growth hormone preparations from different species of animals

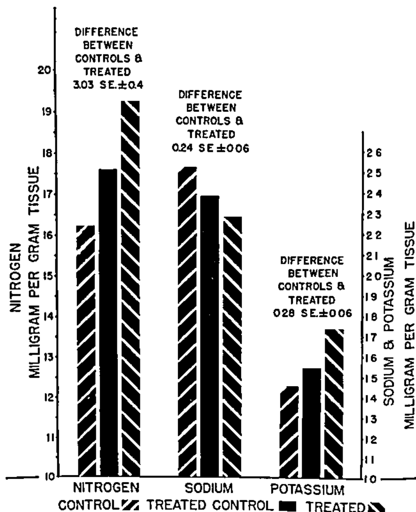


FIG 4 Differences between control and treated pledgets (N Na and K per gram of tissue)

Thus the nitrogen and potassium which are intracellular constituents show a marked increase in the pituitary growth hormone treated pledgets while sodium which is primarily extracellular decreases The differences between the treated and untreated pledgets in the same animal indicate a local as well as systemic effect of pituitary growth hormone

(8) The solution adjusted to pH 4 5–5 0 as before is centrifuged The supernatant is combined with the first supernatant

(9) The combined supernatants are adjusted to pH 8 5 and 95 per cent ethanol is added to a final concentration of 25 per cent ethanol A crystalline precipitate forms often slightly colored and this is centrifuged off This is a very active but still impure growth hormone preparation With ox glands at this stage the yields average about 6 g /kg of whole fresh glands

The final process of purification is achieved in the following steps

(10) The partially purified growth hormone is dissolved in water at pH 11 and to a concentration of one half per cent Any insoluble material is removed by centrifugation

(11) The solution is adjusted to pH 3 5–4 0 at which point it is clear It is then cautiously made alkaline and any precipitates which form are removed by centrifugation These precipitates have usually been found at pH 4 5 5 5 and 9 1 Most if not all of the colored impurities are removed in this process

(12) The pH of the solution is adjusted to 8 5 and 95 per cent ethanol is slowly added in dropwise fashion to a final concentration of 20 per cent ethanol The clean white precipitate usually not crystalline is centrifuged off suspended in distilled water and lyophilized This is purified growth hormone and the yields from ox glands have been of the order of 2 g /kg of whole fresh glands

This procedure has been applied to whole fresh pituitaries of ox sheep pig and fish and to an acetone powder of pig pituitaries Growth hormone preparations of satisfactory activity have been obtained in every instance but one As yet it has been impossible to obtain final products of near homogeneity and of high activity from either fresh pig glands or acetone powders This applies not only to the procedure outlined above but to that of Wilhelmi Fishman and Russell and to any of the intermediate procedures The success of the procedure in processing ox sheep and fish glands suggests that growth hormones of these species and their attendant proteins may have quite similar physical and chemical properties The failure to obtain a highly purified and active pig growth hormone by this method may mean that the active principle in this species is not like that of the others

The biological activities of some of the various preparations have been tested in a number of different ways In Table 1 is summarized a series of observations of weight gain in the hypophysectomized rats which observations were made over a dose range for a number of different preparations Our single preparation of sheep growth hormone compares favorably in activity with the ox preparations Pig growth hormone prepared by the Raben Westermeyer method is somewhat less active The pig material made by our procedure is of a very low order of activity The growth preparations from horse pituitaries were made by Dr Reid who used a modification of the original Li method[1] They have the same order of activity as the

This work has been greatly assisted by a number of able and enthusiastic collaborators Dr Jane Russell Dr Eric Reid Dr Grace Pickford of the Bingham Oceanographic Laboratory at Yale who collected the fish pituitaries used in this work and who made the observations on fish Dr George Friedlander Mrs Joan Altrock Mr Stewart Howard Miss Helen Blair Dr George Adrouny and Mr Raymond Owings all have rendered valuable technical assistance The continuing support of the work by funds granted by the U S Public Health Service and the American Cancer Society is gratefully acknowledged

In most instances the active growth hormone preparations have been made from fresh frozen whole pituitary glands Generous supplies of some of these materials (horse and sheep pituitaries) were given us through the kindness of Mr Irby Bunding and Dr Sanford Steelman of the Armour Research Laboratories Dr C E Graham of the Wilson Laboratories kindly furnished us with fresh whole pig glands with one of their Raben Westermeyer preparations and with a large sample of the acetone powder of pig pituitaries from which that preparation was made

The method by which most of the growth hormone preparations were made differs considerably from that outlined by Fishman Wilhelmi and Russell accordingly it is briefly outlined as follows

(1) The fresh frozen whole pituitaries are mixed with solid carbon dioxide and ground to a fine powder

(2) After the carbon dioxide has evaporated the cold mush is suspended in 0 3 *M* KCl solution (5 ml /g of fresh glands) the pH of the mixture is adjusted to 5 5 and the extract is stirred mechanically overnight This and all subsequent steps are carried out in a cold room at 0–5° centigrade

(3) The extract is centrifuged and cleared if necessary of floating particles by passing it through a glass wool plug and the pH is adjusted to 8 5

(4) Ninety five per cent ethanol is slowly added dropwise to the vigorously stirred extract and in an amount sufficient to bring the ethanol content to 10 per cent The precipitate is centrifuged off and discarded

(5) Ninety five per cent ethanol is added to the extract as before until the ethanol concentration is 30 per cent A precipitate forms which contains the greater part of the growth hormone of the original extract It is a crude growth hormone concentrate

The crude concentrate is further purified in the following steps

(6) The precipitate is redissolved in 0 1 *M* KCl at pH 11 such that a final concentration of one per cent protein is reached

(7) The pH of the solution is adjusted to 4 5–5 0 that pH being selected at which flocculation of the precipitate is most complete The precipitate is centrifuged off the supernatant is decanted and set aside and the precipitate is redissolved at pH 11 in half the original volume of 0 1 *M* KCl

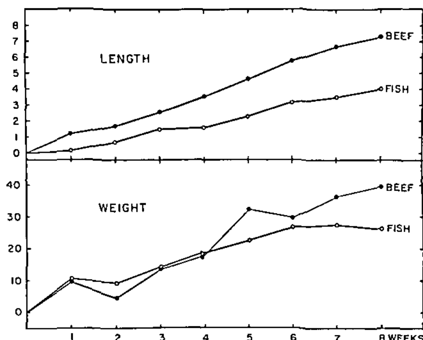


FIG 1 Effect of ox and of fish (pollack) growth hormone on weight and length in the hypophysectomized killifish *Fundulus heteroclitus* From G E Pickford *Endocrinology* 55 274 (1954)

Two pig preparations and 2 fish preparations have been compared with ox growth hormone in respect to the retention of administered amino acid nitrogen in the nephrectomized rat which procedure Dr Russell has described in a preceding paper By this test both the Raben Westermeyer pig preparation and one of our own were about equally active but they were only about one tenth as active as the ox hormone used as a standard The first fish preparation was not only inactive but also somewhat toxic No effect was obtained with the second fish preparation when it was given at a dose level of 1 mg per rat or slightly over 3 times the dose of the standard ox hormone required to give a maximal response

Two series of observations have been made with fish growth hormone studying its effect on the level of cardiac glycogen in the rat during the first 12 hours of a fast These are summarized in Table 2 in which the data on the response to ox growth hormone are presented for comparison At a total dose of 1 mg per 100 g of body weight the fish (pollack) hormone produced a small increase in cardiac glycogen over that of the controls but the mean difference is not significant When the hormone was given at a dose of 2 mg per 100 g no significant effect on the cardiac glycogen was observed

Raben Westermeyer pig preparation The fish growth hormone was quite inactive in rats In the series of tests with the fish hormone it was noted that a weight gain occurred during the first five days of the test but that all of this increment and occasionally more, was lost during the last 5 days We thought it possible that a shorter test such as the tibia test might reveal that the material was active Accordingly, the material was administered to a series of rats for 4 days at a dose level of 2 mg /day In 2 such tests the epiphyseal widths averaged 114 and 133 micra values which are not different significantly from the control values An active preparation of ox growth hormone at a dose of 20 micrograms per day increased the epiphyseal width to 249 micra

Table 1

GROWTH RESPONSE TO OX HORSE SHEEP PIG AND FISH GROWTH HORMONES

Source	*Daily Dose—Micrograms*			
	20	*50*	*100*	*200*
Ox	15 (9)	18 (8)	20 (3)	29 (3)
Horse	10 (3)	8 (1)	11 (2)	
Sheep		21 (1)		29 (1)
Fish	−1 (2)		3 (1)	−1 (2)
Pig (rw)		12 (3)		
Pig (w)		1 (1)		5 (1)

Weight gain or loss in g /10 days
(rw) = Raben Westermeyer growth hormone
(w) prepared by method described in this paper

The assurance that we had in fact prepared fish growth hormone was gained from tests on the killifish *Fundules heteroclitus* which tests were carried out by Dr Grace Pickford Our first preparation derived mainly from hake pituitaries induced gains in the weight and length of normal fish when it was injected at the rate of 300 micrograms weekly and given during the season in which these fish are normally static The second preparation a somewhat purer material derived from pollack pituitaries induced gains in the weight and length of hypophysectomized killifish when the hormone was administered in amounts of 300 micrograms per week for a period of several weeks Data from the latter experiment are illustrated in Figure 1 taken from Dr Pickford s published account [4] It will be noted that the fish hormone was somewhat less active than the ox hormone This may have been due to the presence in the ox hormone of thyrotropin (TSH) in an amount just sufficient to maintain a normal thyroid state in the treated fish The fish pituitary preparation was entirely free of TSH and it appeared also to be free of gonadotropic and adrenocorticotropic activity

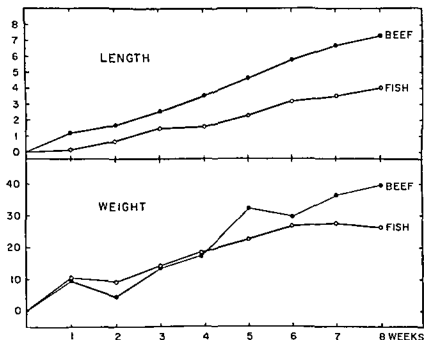


FIG 1 Effect of ox and of fish (pollack) growth hormone on weight and length in the hypophysectomized killifish *Fundulus heteroclitus* From G E Pickford *Endocrinology* 55 274 (1954)

Two pig preparations and 2 fish preparations have been compared with ox growth hormone in respect to the retention of administered amino acid nitrogen in the nephrectomized rat which procedure Dr Russell has described in a preceding paper By this test both the Raben Westermeyer pig preparation and one of our own were about equally active but they were only about one tenth as active as the ox hormone used as a standard The first fish preparation was not only inactive but also somewhat toxic No effect was obtained with the second fish preparation when it was given at a dose level of 1 mg per rat or slightly over 3 times the dose of the standard ox hormone required to give a maximal response

Two series of observations have been made with fish growth hormone studying its effect on the level of cardiac glycogen in the rat during the first 12 hours of a fast These are summarized in Table 2 in which the data on the response to ox growth hormone are presented for comparison At a total dose of 1 mg per 100 g of body weight the fish (pollack) hormone produced a small increase in cardiac glycogen over that of the controls but the mean difference is not significant When the hormone was given at a dose of 2 mg per 100 g no significant effect on the cardiac glycogen was observed

Table 2

EFFECT OF GROWTH HORMONE ON RAT HEART GLYCOGEN

	Number of Observations	*Glycogen (mg %)*
Controls	8	325 ± 36*
Fish 1 mg	8	470 ± 27
Fish 2 mg	6	340 ± 24
Ox 0 25 mg	6	430 ± 27
0 5 mg	6	499 ± 38

* Mean and standard error
The fraction of the total glycogen that is extractable with trichloroacetic acid is measured

A series of preparations from ox pig fish horse and sheep pituitary glands were hydrolyzed in 6N hydrochloric acid One dimensional paper chromatograms were run on the hydrolysates using various solvent mixtures Figure 2 representing a chromatogram run in *n* butanol/formic acid/water is typical of the results obtained No gross differences in amino acid composition were observed

The dinitrofluorobenzene method of Sanger [6,7] was applied to the preparations of ox horse pig and fish (hake) pituitaries The runs were made on a small scale using about 5 mg of protein because of the limited amounts of material which were available Only the major constituents of the DNP mixture could be identified and considerable losses of material occurred [7] DNP alanine and DNP phenylalanine in approximately equal amounts were yielded by each of the hormone preparations examined The quantitative data were not accurate enough to establish that there were significant variations between the different preparations (Table 3) Since small amounts of protein were used and lo ses were large it is possible that other end groups were present but were missed The content of amide N in the different preparations determined in another series of experiments was about the same in each instance

A number of the preparations have been subjected to paper electrophoresis at several different hydrogen ion concentrations There were two series of observations One was made with ox horse and fish (hake) growth hormone and in a constant temperature room at 24 C the second was made with two fish growth hormone preparations (hake and pollack) in a cold room at 10 C In the first series of observations all of the preparations behaved alike with respect to mobility and homogeneity There were indications at some pH values that all of the preparations were to some extent heterogeneous but in no case did a separate spot appear When the movement of the spots from the origin was compared with that of a neutral marker (fructose sucrose or inulin), it appeared that the growth hormones

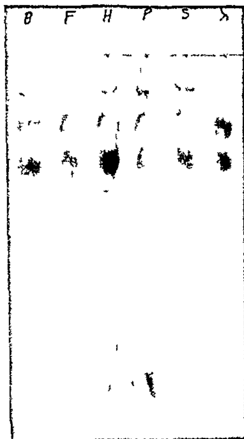


FIG 2 One dimensional paper chromatogram of amino acids in several hydrolyzed growth hormone preparations Amino acid mixture applied at x in amounts equivalent to about 0 2 mg of original protein Solvent *n* butanol/formic acid/water Ninhydrin stain B—ox F—fish (hake) H—horse P—pig (Raben Westermeyer) S—sheep Each was hydrolyzed for 10 hours in 6N HCl

Table 3

FREE AMINO NITROGEN AND AMIDE NITROGEN IN OX FISH HORSE PIG AND SHEEP GROWTH HORMONES

Source	*Per cent α amino N (alanine phenyl alanine)*	*Per cent ε amino N (lysine)*	*Per cent Amide N*
Ox	0 03	0 6	0 70
Fish	0 025	0 7	0 65
Horse	0 035	0 6	0 70
Pig (rw)	0 025	0 6	0 75
Sheep	0 01	0 7	0 75

(rw) = Raben Westermeyer preparation

were not moving freely with the electroendosmotic flow of the buffer be cause possibly they tended to strongly adsorb on the paper The measure ment of *mobilities was therefore not very certain* At best it may be said that both horse and hake growth hormones have isoelectric points somewhat below that of the ox hormone the fish protein being the lower of the two In Figure 3 is shown a representative observation from this series a glycine buffer of pH 10 2 was used The second series of observa tions made on hake and pollack hormones revealed (a) that the hake prepa ration is considerably less homogeneous than that from pollack glands and (b) that the isoelectric point of the pollack growth hormone lies between pH 6 2 and 7 2 This is more nearly in the neighborhood of the isoelectric



Fig 3 Paper electrophoretic diagram of purified growth hormone prepara tions in glycine—NaCl buffer pH 10 2 Whatman #1 paper potential gradient *20v/cm* after 4 5 hours at 25° C B and B ox growth hormone P pig (Raben Westermeyer) F fish (hake) and H horse Protein applied at solid line stained with bromphenol blue Final position of neutral markers at dotted line

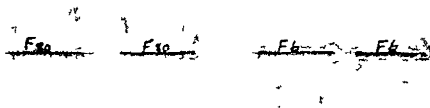


FIG 4 Paper electrophoretic diagram of fish growth hormone preparations in 0 1 *M* phosphate buffer pH 6 2 Whatman #1 paper potential gradient 20v/cm after 16 hours at 10 C F_6 hake F_{50} pollack

point of the ox hormone while as observed before the hake hormone is a more acid protein In Figure 4 is presented an observation on the electro phoretic mobilities of the hormones in 0 1 *M* phosphate buffer and at pH 6 2 and the point at which the two preparations moved in opposite directions is apparent

Using the moving boundary method a few observations were made on beef fish (hake) horse and pig growth hormones at pH 6 3 and on fish and sheep hormones at pH 5 8 Only small amounts of material were available and since the solubilities of the preparations were low at these pH values the protein concentrations were only about 0 2 per cent At pH 6 3 the main components of the fish horse and pig preparations migrated toward the anode at pH 5 8 the main component of the sheep preparation was stationary while that of the hake hormone still migrated toward the anode These observations are necessarily only of a qualitative character but they indicate that pig horse sheep and hake growth hormones are all somewhat more acid proteins than are ox and pollack hormones

Ox horse hake and pollack growth hormone preparations have been examined in the Spinco ultracentrifuge Model E Runs were made at about 60 000 r p m using a glycine buffer of ionic strength 0 1 at pH 9 7 In Figure 5 are illustrated 3 runs with ox (top) hake (middle) and horse

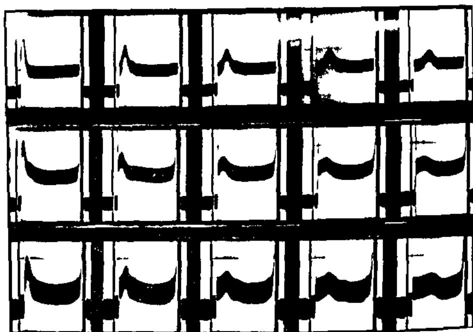


FIG 5 Sedimentation diagrams for ox (top) fish (hake middle) and horse growth hormones in glycine NaCl buffer pH 9 5 Photographs taken at 16 min intervals after reaching maximal speed (about 60 000 r p m)

growth hormones the photographs having been taken at 16 minute intervals It will be noted that each preparation contains a small heavy impurity that the hake preparation sediments much more slowly than the others and that horse and ox preparations sediment at about the same rate Hake and pollack hormones were compared in a later series of observations The estimates of the sedimentation constants for these runs are presented in Table 4 The value for ox hormone is somewhat lower than that reported by other workers [6 8 9 10] Both hake and pollack growth hormones have significantly lower sedimentation constants than have the ox and horse hormones

Table 4

ULTRACENTRIFUGATION OF OX FISH AND HORSE GROWTH HORMONES

Source	*% Protein*	*$S_{20} \times 10^{-3}$ (cor)*
Ox	0 6	2 72
Fish		
Hake	0 6	1 76
Hake	1 0	2 15
Hake	1 0	1 43
Pollack	1 0	1 78
Horse	1 2	2 71
Horse	0 6	2 96

These rather scattered observations do little more than outline the problem of the comparative study of the growth hormones of different species of animals Their main value at the moment consists in providing better direction to the isolation and purification of the hormones In the light of the newer information about their isoelectric points and molecular sizes it should be possible to redesign the methods of preparation If the limitations of source material can be overcome and if the methods of isolation can be adapted specifically the very pure preparations can be obtained in amounts adequate to permit sound detailed chemical and physical analyses

The differences among the growth hormones of different species are already very striking It is most interesting that fish growth hormone is inactive in mammals i e in the limited number of tests so far employed whereas ox growth hormone is active in fish This finding may be related to the fact that the molecules are of very different size and possibly of fundamentally different structure There cannot be too radical a difference however since the ox hormone is active in fish and must be a protein as foreign to the fish as the fish protein is foreign to the mammal The difference in isoelectric points among the various mammalian preparations also indicates differences in composition and possibly in structure Yet these are also active in the rat the common mammalian test animal It is possible that a detailed examination of relative activities over a wider range of the actions ascribed to growth hormone will reveal in fact differences which can be related to the observed differences in chemical and physical properties

It is also possible however that all of the growth hormones possess a common nuclear structure which is responsible for their activity and that the detailed differences in the rest of the molecule are peculiar to a given species This may be sufficient to result in marked relative differences of activity in the interspecies tests The detailed analysis of the different highly purified growth hormones may provide the answer to this problem

References

1 Li C H Evans H M and M E Simpson *J Biol Chem* **159** 353 (1945)
2 Wilhelmi A E Fishman J B and J A Russell *J Biol Chem* **176** 735 (1948)
3 Raben M S and V W Westermeyer *Proc Soc Exp Biol Med* **78** 550 (1951)
4 Pickford G E *Endocrinology* **55** 274 (1954)
5 Sanger F *Biochem J* **39** 507 (1945)
6 Reid E *J Endocrinology* **9** 210 (1953)
7 Li C H and L Ash *J Biol Chem* **203** 419 (1953)
8 Li C H and M J Moskowitz *Biol Chem* **178** 203 (1949)
9 Smith E L Brown D M Fishman J B and A E Wilhelmi *J Biol Chem* **177** 305 (1949)
10 Li C H and K O Pedersen *J Biol Chem* **201** 595 (1953)

5

Hypophyseal Growth Hormone as a Protein*

Choh Hao Li Hubert Clauser † Peter Fønss Bech ‡ A L Levy,§ Peter G Condliffe ¶ and Harold Papkoff

Hormone Research Laboratory University of California Berkeley

Introduction

In 1939 van Dyke[1] wrote No investigator has succeeded in preparing a satisfactorily pure growth promoting extract of the pituitary body There is general agreement that the pituitary among endocrine glands is the most important regulator of growth but whether this regulation is effected by a specific *growth promoting* hormone or by direct or indirect combined effects of other pituitary hormones such as the lactogenic and the thyrotropic hormones remains an undecided issue The only sensible verdict to render in answer to the plea that the anterior pituitary elaborates (or does not elaborate) a specific growth promoting hormone is the Scotch verdict of *not proved*

In 1944 a homogeneous protein possessing growth promoting activity was prepared in this laboratory from the alkaline extract of ox anterior pituitaries and at that time we believed the protein to be the hormone [3] The object of this paper is to review the evidence which has accumulated for the last few years in support of this contention The chemical composition of this hormone protein will also be discussed and certain structural studies will be outlined

---

* Grants in aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council and from the Albert and Mary Lasker Foundation have made these studies possible

† Present address Laboratoire de Chimie biologique Faculte des Sciences Universite de Paris

‡ Present address Lind Holmsvej 19 Copenhagen Denmark

§ Deceased August 21 1954

¶ Present address National Institute of Arthritis and Metabolic Disorders National Institutes of Health Bethesda Md

Method of Isolation

The first published method for the isolation of growth hormone yielded only 0 04 g from one kilogram of the anterior lobe of ox pituitaries [4] Later Wilhelmi and his colleagues developed an ethanol fractionation technique which gave rise to a crystalline product in considerably improved yield In the course of the past few years our original procedure has been simplified and the yield increased 50 fold [6] All of the steps outlined below are carried out at 1 C

One kilogram of anterior lobes of ox pituitaries is ground and stirred vigorously with 2 liters of $Ca(OH)$ solution at pH 10 3 for about one hour and is then kept frozen for at least 5 or 6 hours After being thawed the mixture is centrifuged in a Spinco L shaped ultracentrifuge at a speed of 20 000 RPM and the residue washed with 1 liter of $Ca(OH)$ solution at pH 10 3 The supernatant fluid and the washing are combined and brought to 0 5 saturation with respect to ammonium sulfate by the addition of solid $(NH_4)\ SO_4$ The precipitate formed is dissolved in 500 ml of water and dialyzed against running water for 16 to 20 hours After dialysis a saturated ammonium sulfate solution is added until the concentration of $(NH_4)\ SO_4$ is brought to 0 2 saturation and the mixture is adjusted to pH 6 8 The precipitate formed is centrifuged and discarded The supernatant fluid is brought to 0 4 saturation by the addition of more saturated ammonium sulfate solution at pH 6 8 and the precipitate which forms is dissolved in water and dialyzed thoroughly until salt free The insoluble material inside the dialysis bag is dissolved in 200 ml of water with the aid of 1 0 *M* HCl The clear acidified solution is adjusted to pH 5 3 with 1 0 *M* NaOH The precipitate formed is removed by centrifugation and discarded The pH of the supernatant fluid is next increased to 6 8 and the flocculent precipitate which occurs is dissolved in 100 ml of water adjusted to pH 10 5 by the addition of 1 0 *M* NaOH The resulting clear solution is adjusted to pH 8 7 with 1 0 *M* HCl After centrifugation the supernatant fluid is frozen and dried in a vacuum The lyophilized product (*Fraction A*) weighs 3 5 g

For the next step 3 5 g of Fraction A are dissolved in 350 ml of water adjusted to pH 4 0 by the addition of 1 0 *M* HCl To this a saturated solution of NaCl is added dropwise to 0 016 saturation The precipitate which forms is discarded after centrifugation An equal volume of saturated NaCl is added slowly to the supernatant fluid The precipitate is then dissolved in 200 ml of water adjusted to pH 10 0 by the addition of 1 0 *M* NaOH and dialyzed against distilled water for 6 hours with frequent change of the dialyzate The dialyzed solution is adjusted to pH 8 7 if precipitation occurs the solution is centrifuged and the precipitate discarded The pH is then adjusted to 6 8 by the addition of 0 1 *M* HCl After centrifugation the precipitate is dissolved in 150 ml of water adjusted to pH 9 0 by the addi

tion of 1 0 *M* NaOH and lyophilized the product is called *Fraction B* The supernatant fluid at pH 6 8 is next brought to an ethanol concentration of 20 per cent by adding very slowly an equal volume of 40 per cent ethanol The precipitate which forms is dissolved in 100 ml of water adjusted to pH 9 0 by the addition of 1 0 *M* NaOH and the solution is then frozen and dried in a vacuum the lyophilized white powder is designated as *Fraction C* The yields of Fractions B and C amount to 1 4 g and 0 5 g respectively

Physicochemical and biological investigations have revealed no differences between Fractions B and C Thus this simplified procedure yields on the average of 2 g of the growth hormone from 1 kilogram of ox anterior lobes of the pituitary glands

The biological potency of the preparation as determined by the tibia width assay method[7] is discussed elsewhere in this volume[8] In hypophysectomized female rats (operated upon at 28 days and injected 14 days postoperatively) a daily dose of 0 01 mg caused a 12 g increase in body weight in a period of 10 days (Table 1) Hence the growth promoting activity of the hormone isolated by the simplified procedure is practically identical to that of the preparation reported earlier[4]

Table 1

BIOASSAY OF GROWTH HORMONE ACCORDING TO BODY WEIGHT INCREMENT PRODUCED IN HYPOPHYSECTOMIZED RATS

Daily Dose (mg)	*No of Rats**	*Body Weight (mean ± S E)* *Onset (g)*	*Final (g)*	*Gain in 10 Days (g)*
0 00	10	63 ± 0 9	64 ± 1 6	1
0 01	11	65 ± 1 7	78 ± 2 6	13

* Hypophysectomized female rats (28 days old at operation) were injected intraperitoneally with 0 5 ml of solution once daily for 10 days beginning on the 14th postoperative day

Homogeneity Investigations

The difficulties in defining the purity of a protein are well known and have been discussed by a number of investigators[9] It is generally agreed that if there is *uniformity* with respect to physicochemical and biological properties among various preparations of a protein it is highly probable that the protein is pure Isolated criteria of purity are not sufficient and even agreement among a large number of criteria does not insure purity but only increases the probability a single new criterion may perhaps show that the protein preparation is in fact a mixture For the last few years a number of new techniques have been developed to investigate the homogeneity of proteins these include adsorption chromatography zone electrophoresis counter-current distribution end group analyses etc Hence re-examination

of the purity of growth hormone preparation in terms of these advances would seem desirable

Ultracentrifugation[10 11] All the preparations of growth hormone which have been investigated in both the Spinco and the Svedberg oil turbine models of analytical ultracentrifuge have given evidence of being monodisperse (Fig 1) However there are indications that the rate of sedimentation (S_0) depends upon the pH as well as the concentration of the hormone protein[11] At pH 9 93 the variation of S_0 in relation to protein concentration (C per cent) may be represented by the equation

$$S_0 = 3\ 19 + 0\ 22\ C$$

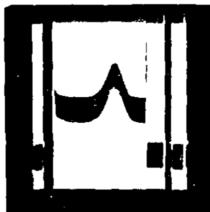


Fig 1 The schlieren pattern of growth hormone in a borate buffer of pH 9 5 and 0 1 ionic strength containing 0 1 *M* NaCl taken 74 minutes after centrifugation at 59 780 r p m at room temperature in a Spinco ultracentrifuge

Boundary Electrophoresis[12] Electrophoretic investigations of growth hormone preparation in an acetate buffer of pH 4 0 and 0 03 ionic strength in the Perkin Elmer model of the Tiselius apparatus have been made routinely for the last few years in this laboratory In every case a single boundary was obtained indicating a high degree of electrophoretic homogeneity Studies in buffers of different pH s confirm what was observed at pH 4 0 (Fig 2)

Zone Electrophoresis[13] It is entirely possible in connection with boundary electrophoresis of biologically active proteins that a single boundary may not necessarily represent the *actual* active component One of the main difficulties in the conventional Tiselius apparatus is in obtaining fractions from the U tube on both sides of the boundary Moreover it is only the fastest moving component that can be obtained in a form free from other components It is particularly difficult to employ this technique for homogeneity studies if the active protein consists chiefly of an inert material with only a very small amount (1 or 2%) of the highly active component The

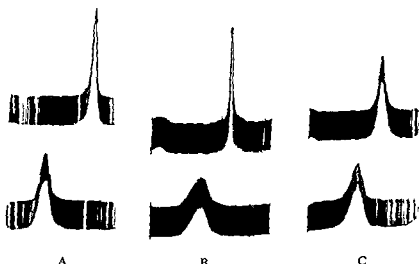


Fig 2 Electrophoretic patterns of growth hormone (1% solution) (A) glycine buffer of pH 3 5 and 0 1 ionic strength 7200 seconds at a potential gradient of 5 8 volts/cm (B) acetate buffer of pH 4 0 and 0 03 ionic strength 3000 seconds at a potential gradient of 12 3 volts/cm (C) glycine buffer of pH 11 5 and 0 06 ionic strength 2700 seconds at a potential gradient of 11 3 volts cm

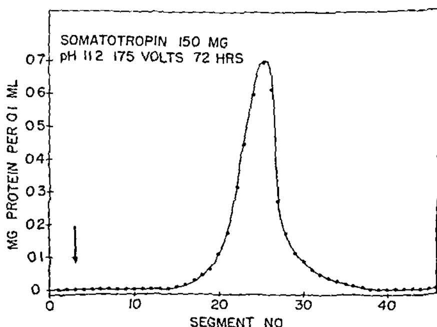


Fig 3 Zone electrophoresis of growth hormone on starch in carbonate solution at pH 11 2 Taken from Fønss Bech and Li [13]

recent development of the technique of *zone* electrophoresis appears to have overcome these difficulties since by the latter method each component no matter how low its concentration will migrate as a discrete zone along the inert support In addition each individual zone can be isolated and its chemical and biological characteristics investigated separately

When growth hormone preparations were submitted to zone electrophoresis with starch as the supporting medium it was found that in every case the hormone protein migrated as a single zone (Fig 3) It is significant that the amount of material originally applied to the trough could be accounted for by the protein recovered from the peak and that the growth promoting activity of the fractions recovered from the entire peak was not found to differ significantly from that of the starting material Further the peak was divided into three fractions whose biological activity did not differ significantly one from another (Table 2) These results strongly suggest that the protein may be the hormone

Table 2

BIOASSAY OF SOMATOTROPIN FRACTIONS OBTAINED FROM ZONE ELECTROPHORESIS

Electrophoresis		*Fraction*	*No of Rats**	*Width of Uncalcified Cartilage of Tibia* (μ)
pH	*Buffer*			
4 0	Acetate	Whole peak	5	218 ± 5 5† (>0 3)‡
9 0	Carbonate	"	6	237 ± 3 2
11 2	"		5	230 ± 2 5
11 2	"	Peak left side	10	215 ± 4 5 (>0 1)
11 2		top	10	235 ± 3 9
11 2	"	right side	10	221 ± 3 0
Starting material			21	224 ± 3 2

Each animal received a total dose of 80 γ in 4 days
† Mean ± standard error
‡ *p* values from Fisher s table of *t*

Solubility [14] There are two procedures that are generally employed for studies of the degree of homogeneity of proteins as determined by their solubility one introduced by Northrop [1] depends upon the principle of phase rule [16] and the other suggested by Cohn [17] involves solubility characteristics as the function of salt concentration We reported previously[4] results obtained with the Northrop procedure wherein the solubility of the hormone protein was found to be constant after the appearance of the solid phase (Fig 4) Recent studies of the solubility of growth hormone in (NH_4) SO_4 solutions using the Cohn technique have provided further

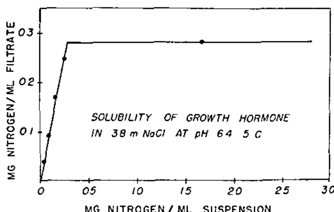


FIG 4 Solubility curve of growth hormone at 5 C solvent 3 8 M NaCl in 0 07 M phosphate buffer at pH 6 4 Taken from Li Evans and Simpson[4]

evidence for the homogeneity of the hormone protein A typical experiment may be seen in Figure 5 It is evident that the hormone protein behaves like other homogeneous proteins in that a straight line relationship exists when the salt concentration is plotted against the logarithmic function of the solubility as defined by Cohn[17]

$$\log S = \beta - K\mu$$

where μ represents the ionic strength S the solubility of the protein in g per liter, and K and β characteristic constants for the protein under investigation

The data in Figure 5 were obtained from studies of the solubility of growth hormone in ammonium sulfate solutions 1 0 ml of a 1% aqueous solution of growth hormone was added to 9 0 ml of a buffer solution Buffer solutions were prepared by mixing saturated $(NH_4)_2SO_4$ solution

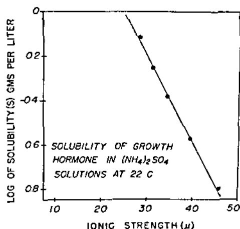


FIG 5 Solubility of growth hormone in $(NH_4)_2SO_4$ solutions at 22 C

in the appropriate volume for different concentrations with 25 ml of citrate buffer of pH 5 7 and 0 2 ionic strength and sufficient water to make a total of 50 ml The protein solutions were kept at 22° C for 24 hours with occasional stirring After centrifugation the protein concentration was estimated from the UV absorption at 275 mu in a Model DU Beckman spectrophotometer

Adsorption Chromatography [18] Although the adsorption and elution of proteins have been subjects of study for many years it is only recently that adsorption chromatography has been used for the investigation of the purity of proteins [19] While basic proteins of low molecular weight have been chromatographed successfully by means of both elution and partition techniques proteins of high molecular weight because of the irreversibility of their adsorption have been subjected to chromatographic investigation without much success [0] We have recently discovered[18] that the hormone protein can be adsorbed onto a Hyflo Super Cel® column with buffers of a pH below the isoelectric point and that the adsorbed protein can be eluted as a single peak by increasing in either a continuous or discontinuous gradient, the pH of the developing solvent No loss of biological activity was observed after the hormone had been repeatedly submitted to this procedure of adsorption and elution It was further demonstrated that the hormone derived therefrom exhibits chromatographic homogeneity

An example of a characteristic experiment in which continuous gradient elution was employed is presented in Figure 6 A column containing 30 g of Hyflo Super-Cel was used the starting buffer was a citrate buffer at

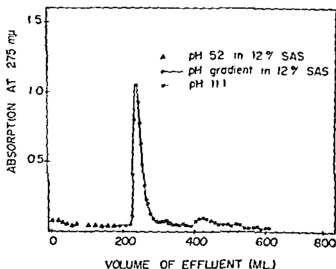


Fig 6 Adsorption and elution of growth hormone (47 mg) on a 30 g Hyflo Super-Cel® column with a continuous pH gradient Taken from Clauser and Li [18] SAS—saturated $(NH_4)_2SO_4$

Table 3

BIOASSAY OF SOMATOTROPIN BEFORE AND AFTER ADSORPTION CHROMATOGRAPHY

Experiment No	Diameter of the Column (cm)	Amount of Hormone Employed (mg)	Material Recovered (%)	Bioassay by the Tibia Test*			
				Before Chromatography		After Recovery from Column	
				No of Rats	Tibia Width (micra)	No of Rats	Tibia Width (micra)
4†	1	8 5	80	7	211 8 ± 2 9‡	5	206 8 ± 4 3
5	1	6 6	91	7	216 1 ± 4 2	6	217 8 ± 2 7
7	1	5 4	88	7	225 2 ± 5 3	7	224 4 ± 1 7
12§	2 3	47 0	84	6	233 5 ± 2 6	I 6	225 5 ± 3 7
						II 5	242 5 ± 1 7
14–15	3 1	84 0	78	12	234 ± 2 0	15	237 ± 1 7
16	3 1	90 0	75	12	224 ± 3 5	11	226 3 ± 2 8
17	3 1	84 0	68	12	224 ± 3 5	12	223 5 ± 2 7
19	2 3	33 6¶	81	6	230 ± 3 4	7	226 ± 5 8

* Animals given a total dose of 80 μg in 4 days Those receiving saline had an average tibia width of 155 μ

† This experiment was carried out with a discontinuous pH gradient all others were conducted by the continuous gradient procedure

‡ Mean ± standard error

§ In this experiment the peak was divided into two fractions I represents the left portion and II the right Each fraction was assayed separately

¶ The hormone was recovered from Experiment No 17

pH 5 2 containing 12% saturated $(NH_4)_2SO_4$ After 47 mg of the protein hormone had been introduced onto the column the same buffer in a total volume of approximately 200 ml was allowed to run through At the end of 6 hours the continuous pH gradient was started with buffers of higher pH as the pH gradually shifted a sharp peak representing the hormone protein appeared in the pH range between 6 8 and 8 1 reaching its maximum at pH 7 5 The peak was divided into 2 fractions and assayed in hypophysectomized rats It may be seen in Table 3 that 85% of the material appeared in the main peak and that the activity of both shoulders was not significantly different from that of the starting material Other experiments summarized in Table 3 demonstrated that 80–90% of the protein could be recovered as a single peak and furthermore that the hormonal activity of the protein recovered from the peak is practically identical with that of the hormone protein before chromatography Thus no fractionation of the hormone protein or separation of biological activity from the protein occurs in these adsorption experiments This may be taken as additional evidence for the purity of growth hormone preparations

Counter current Distribution The recently developed counter current distribution technique of Craig [1] has been established as one of the most powerful tools for the study of the purity of organic compounds of low molecular weight One of the best examples[2] of its application was the demonstration of crystalline insulin as a mixture of insulin A and insulin B these two insulins differ from each other only by one amide group [3] Because of surface denaturation it has not been found successful to employ this technique for investigations of proteins whose molecular weight is higher than 10 000 However we have succeeded recently in distributing lactogenic hormone (m wt = 25 000) in a solvent system consisting of aqueous dichloroacetic acid and 2 butanol [4] Unsuccessful attempts to investigate the behavior of growth hormone preparations in counter current distribution have been made for the last two years in our laboratory it is therefore gratifying that Pierce has very recently reported solvent systems for distribution studies on growth hormone

Pierce has investigated the partition of a sample of our growth hormone preparations between 2 butanol and approximately 0 005 *M* *p* toluene sulfonic acid In this solvent system through 24 transfers it was found that over 97% of the material has a partition coefficient of approximately 10 (Fig 7) and that growth promoting activity was present in the major component the distribution curve of which agreed well with the theory for a single substance Although it is recognized that the most ideal partition coefficient for purity studies in counter current distribution should be near unity and that many more transfers than 24 are required for such investigations the experiments of Pierce at least suggest that the protein is associated with the hormonal activity and is not separated from it by the partition procedure

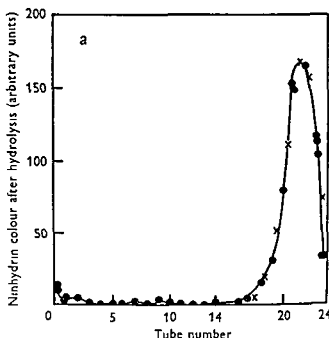
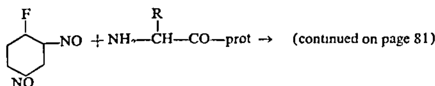


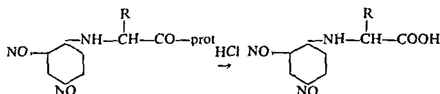
FIG 7 Distribution curve of 22 mg of growth hormone after 24 transfers Solvent system 2 butanol/approx 0 005 *M* *p* toluenesulphonic acid ● arbitrary units of color density multiplied by 100 with correction made for dilution of samples X theoretical values Taken from Pierce [5]

N terminal Amino Acid Analysis If a protein has a structure consisting of *one* peptide chain and if the peptide chain is *not* cyclic there should be two terminal residues one with a free amino group and the other with a free carboxyl group The number of terminal amino groups corresponds to the number of peptide chains in the protein these chains are held together by the —S—S— bridge to form the protein molecule A pure protein should therefore yield quantitatively one or two moles of N terminal residue(s) per mole of the protein according to the number of peptide chains in the molecule Based upon these premises terminal residue analysis may provide additional information about the homogeneity of a protein

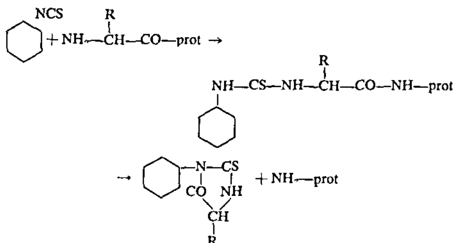
The two available methods for the analysis of the N terminal residue in proteins or peptides are the fluorodinitrobenzene (FDNB) procedure of Sanger [6]



(continued on page 81)



and the phenylisothiocyanate (φNCS) reaction of Edman[27]



These two methods have recently been employed for investigations of the N terminal residues in growth hormone[8, 9] The FDNP method gives alanine and phenylalanine as N terminal residues in amounts of 0 8 and 0 9 mole respectively for each mole of the hormone these data were con firmed by the reaction of φNCS with the hormone protein (Table 4) In fact the φNCS procedure indicates that exactly one mole each of the phenylthio hydantoins (PTH) of alanine and phenylalanine was formed per mole of the hormone after the hydrolysis of the phenylthiocarbamyl (PTC) deriva tive of the hormone The occurrence of DNP or PTH phenylalanine and DNP or PTH alanine in a molar ratio of 1 1 in DNP or PTC hormone may be taken as further support for the view that the hormone is a homo geneous protein

Table 4

N TERMINAL RESIDUES IN GROWTH HORMONE

N terminal Residues	*Moles of N terminal Residue/Mole of the Hormone*	
	FDNP Method	*φNCS Method*
Alanine	0 8	1 0
Phenylalanine	0 9	1 0

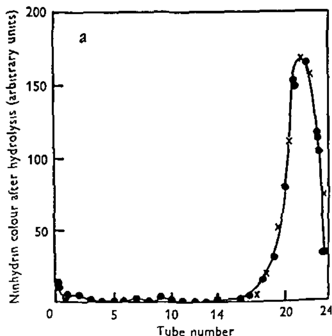
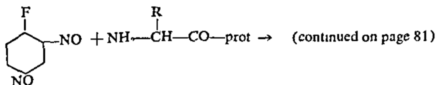


FIG 7 Distribution curve of 22 mg of growth hormone after 24 transfers Solvent system 2 butanol/approx 0 005 *M p* toluenesulphonic acid ● arbitrary units of color density multiplied by 100 with correction made for dilution of samples *X theoretical values Taken from Pierce*

N terminal Amino Acid Analysis If a protein has a structure consisting of *one* peptide chain and if the peptide chain is *not* cyclic there should be two terminal residues one with a free amino group and the other with a free carboxyl group The number of terminal amino groups corresponds to the number of peptide chains in the protein these chains are held together by the —S—S— bridge to form the protein molecule A pure protein should therefore yield quantitatively one or two moles of N terminal residue(s) per mole of the protein according to the number of peptide chains in the molecule Based upon these premises terminal residue analysis may provide additional information about the homogeneity of a protein

The two available methods for the analysis of the N terminal residue in proteins or peptides are the fluorodinitrobenzene (FDNB) procedure of Sanger [26]



(continued on page 81)

Biological Tests The importance of establishing the purity of a biologically active protein is so obvious that it needs no emphasis here A minute impurity can be responsible for some observed effect which may be erroneously attributed to the main component because the impurity quite often alters the course of the biological action of the main component It is fortunate that the biological tests for the contaminants are usually very sensitive and that their presence is therefore easily detected It should always be kept in mind however that a pure substance may possess intrinsically additional biological characteristics other than its known major function Moreover the possibility cannot be excluded that some degree of overlap may occur in the biological activities of chemically similar compounds [30]

One of the most common biologically active contaminants in growth hormone preparations is thyrotropin (TSH) It has been postulated by some investigators that the inability of actually demonstrating the growth promoting effects of growth hormone preparations in man is perhaps due to the TSH contamination We have repeatedly assayed by different methods preparations of growth hormone isolated by the simplified procedure outlined above for possible contamination with TSH and found that one mg of hormone contains less than 0 01 U S P units of TSH (see for example Table 5) Since no one has as yet isolated TSH in pure form and since we do not know how active it is in terms of U S P units we cannot calculate the percentage of contamination of TSH in growth hormone preparations however if one assumes that 1 mg of 'pure TSH represents 1 U S P unit of thyroid stimulating potency the TSH contamination is indeed small

A total dose of 10 mg of growth hormone administered over a period of 4 days gave no histological evidence of the presence of adrenocorticotropic thyrotropic or gonadotropic activity in hypophysectomized female rats (operated upon at 28 days of age and injected 14 days postoperatively) and a total dose of 5 mg injected subcutaneously into month old squabs for 4 days manifested no lactogenic activity A dose of 0 1 mg produced no adrenal ascorbic acid depleting activity in hypophysectomized rats Bioassay for melanophore expanding activity in hypophysectomized *rana pipiens* showed no intermedin contamination with a dose of 0 2 mg When growth hormone preparations were assayed for anti diuretic principle in hydrated rats a dose of 0 5 mg gave no indication of antidiuretic activity From these results it is reasonable to conclude that the growth hormone preparation is essentially free from known biologically active contaminating components other than its own growth promoting activities

Composition

Some years ago we reported the partial amino acid content of growth hormone as determined by microbiological techniques [31] In view of the recent development of chromatographic procedures whereby amino acid

Table 5

ASSAY OF TSH CONTAMINATION IN GROWTH HORMONE

Experiment	*Preparation*	*Dose*	*No of Animals*	*Mean Value*	
				P^{32} Uptake (cts /sec /thyr)	*Acinar Cell Height (micra)*
Chick*	TSH (Armour)	0	4	8 5	
		0 005 U S P unit	5	12 5	
		0 0045 U S P unit	6	18 4	
	Growth hormone	0 001 mg	5	10 8	
		0 010 mg	5	7 7	
		0 100 mg	6	14 2	
Tadpole†	TSH (Armour)	0	4		7 1
		0 005 U S P unit	4		14 2
	Growth hormone	0 5 mg	4		11 6

* Kindly performed by Dr F S Greenspan Stanford University
† Kindly performed by Dr B Catz, University of Southern California

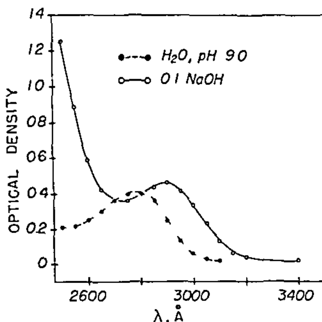


FIG 8 Ultraviolet absorption of growth hormone in pH 9 0 solution and 0 1 N NaOH

method allows an independent determination of the tyrosine content of growth hormone which was found to be 11 5 residues per mole (46 000) The ultraviolet absorption spectrum of growth hormone in aqueous solutions of both pH 9 0 and 0 1 *M* as measured in a Beckman model DU spectrophotometer with a protein solution containing 0 0876 mg N per ml may be seen in Figure 8 The amide N (ammonia) determined by a method of Geschwind[37] was found to be 7 1 g per 100 g protein nitrogen

As it has already been mentioned the growth hormone molecule has two N terminal α amino acids (phenylalanine and alanine) If we assume that it has also two C terminal residues (see below) this information together with the data in Table 6 would provide a basis for computing the acidic

Table 7

IONIC GROUPS OF GROWTH HORMONE

	No of Groups		No of Groups
Aspartic acid	37	Arginine	27
Glutamic acid	53	Lysine	24
Terminal carboxyl	2	Histidine	7
	92	Terminal α amino	2
Amide groups	−30		
Total anionic groups	62	Total cationic groups	60

determinations of high accuracy can be achieved,[32 33 34] it appeared desirable to reinvestigate the complete amino acid composition of growth hormone by one of these new procedures

Table 6

AMINO ACID COMPOSITION OF GROWTH HORMONE

Amino Acid	*Mean Molar Fraction*	*Minimal Molar Ratio*	*No of Residues to the Nearest Integer for M Wt = 46 000*	*g Amino Acid Residual Weight*	*g Amino Acid per 100 g Protein*
Glu	0 1314	26 44	53	6 830	16 27
Asp	0 0921	18 52	37	4 260	10 30
CyS	0 0099	2 00	4	817	2 01
Ser	0 0570	11 48	23	2 000	5 05
Thr	0 0670	13 48	27	2 730	6 79
Gly	0 0532	10 74	21	1 200	3 29
Ala	0 0820	16 52	33	2 345	6 14
Pro	0 0364	7 34	15	1 455	3 61
Val	0 0371	7 48	15	1 485	3 66
Met	0 0175	3 56	7	918	2 18
Leu + Ileu	0 2008	40 44	81	9 160	22 14
Phe	0 0719	14 44	29	4 270	10 00
Tyr	0 0285	5 70	11	1 794	4 16
Lys	0 0593	11 92	24	3 070	7 33
His	0 0177	3 56	7	960	2 26
Arg	0 0681	13 70	27	4 220	9 8[illegible]
Try		(1 00)	2	372	0 85
NH_3			30*		1 12*
Total	1 0279	208 32	416	47 886	115 86

* These values are not included in the totals

The method employed in the present investigation[35] was developed by Levy[34] in this laboratory and is based upon the quantitative paper chromatography of the dinitrophenyl derivatives of the amino acids in the acid hydrolyzate of the protein This method is very sensitive requiring only about 0 2 mg of protein (equivalent to approx 0 1 μM of each amino acid) per chromatogram and attaining an accuracy comparable to that of the classical ion-exchange column procedure of Moore and Stein[33] The quantitative data summarized in Table 6 were obtained by analyses after acid hydrolysis for different lengths of time corrections for losses were made by extrapolation of the recoveries to zero hydrolysis time It is to be noted that leucine and isoleucine cannot be differentiated by the Levy method but previous study[31] has shown that the hormone protein contains both The value for tryptophane was estimated by the ultraviolet absorption of the intact protein according to the procedure of Goodwin and Morton *[36] This

* The author is indebted to Dr I I Geschwind for these determinations

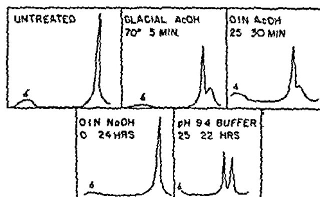


FIG 9 Electrophoretic patterns (ascending boundary) of growth hormone in an acetate buffer of pH 4 0 and 0 03 ionic strength after treatment under various conditions Taken from Li and Papkoff[33]

ever that high temperature and the presence of water are apparently essential for the change of electrophoretic behavior and inactivation As may be seen in Table 9 hormone treated in an acetate buffer of pH 4 0 at 0° C for 5 days remains active with no change of electrophoretic behavior while under the same conditions at 25 C growth promoting activity is greatly decreased with complete conversion into the fast component When a lyophilized sample of the hormone was completely dried over P O in a desiccator and put into an oven of 100 C for 30 minutes there were no changes in either biological activity or electrophoretic behavior

Table 9

INFLUENCE OF VARIOUS CONDITIONS OF TREATMENT ON ACTIVITY OF GROWTH HORMONE

Conditions	*Temperature* (C)	*Time of Treatment* (*min*)	*Bioassay**		*Electrophoretic Components*	
			No of Rats	*Tibia Width* (μ)	*Slow* (%)	*Fast* (%)
Untreated			49	226 ± 2†	100	0
0 01 *N* AcOH	70	30	8	168 ± 8	0	100
0 01 "	100	1	9	158 ± 3	0	100
Acetate buffer pH 4 0	0	7200	6	228 ± 5	100	0
" 4 0	25	4320	13	178 ± 9	15	85
Dry heat	100	30	7	230 ± 5	100	0

* Animals given a total dose of 80 γ in 4 days those receiving saline had an average tibia width of 155 μ

† Mean ± standard error

and basic groups in the hormone protein as given in Table 7 It may be recalled that the isoelectric point of growth hormone as determined in electrophoresis, is located at pH 6 85,[4] which suggests that the number of basic groups may equal that of the acidic groups This is in excellent agreement with the analytical findings (Table 7)

Stability

The stability of growth hormone under various conditions has recently been investigated in some detail [38] When a hormone protein solution is subjected to a variety of treatments obvious changes take place in its physicochemical characteristics One of these changes when the hormone was submitted to electrophoresis in an acetate buffer of pH 4 0 and 0 03 ionic strength at 1 C was the appearance of a new electrophoretic component which possessed a mobility approximately 1 5 $\times$ faster than that of the untreated growth hormone When the duration of treatment was varied *it was observed that the amount of the fast component increased with the time of treatment at the expense of the slow fraction* The rate of conversion depends upon the conditions of treatment In Table 8 is given the time

Table 8

HALF LIFE TIME ($t_{½}$) OF GROWTH HORMONE UNDER VARIOUS CONDITIONS OF TREATMENT

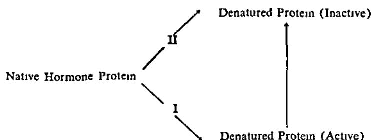
Solvent	*Temperature* (C)	$t_{½}$ (*min*)	*Activity**
Glacial AcOH	70	23	−
0 1 *N* AcOH	25	360	±
Buffer pH 9 4	25	1920	+
0 1 *N* NaOH	25	72	+

* Activity represented as follows − decrease ± doubtful + unchanged Activity was estimated when 50 per cent of the hormone had been converted into the fast component

required to obtain a 50% conversion to the fast component when the hormone protein has been subjected to various conditions of treatment together with information about the biological activity Some of the electrophoretic patterns may be seen in Figure 9 It is significant to note that in 0 1 N NaOH at 25° C 50% of the hormone protein changes into the fast fraction after 72 minutes and yet still remains active whereas in 0 1 *M* acetic acid it takes 360 minutes to convert 50% of the hormone protein into the fast component and the solution is no longer biologically active Although the hormone is known to be a thermolabile protein [39] it is somewhat surprising to find that it is unstable under such mild conditions as for example in buffer solutions of pH 9 4 and at a temperature of 25 C It should be pointed out how

method of any new N terminal α amino groups Moreover the growth promoting activity remains unchanged This catalytic action of chymotrypsin on the denaturation of growth hormone depends upon the concentration of the enzyme As may be seen in Table 11 the percentage of the fast component decreased with the ratio of enzyme/hormone when a 1% hormone solution (pH 8 6) was kept at 25° C for only 60 seconds

From these studies it is evident that growth hormone is very susceptible to denaturation Biological activity alone is not sufficient to be taken as an index of denaturation As shown in Table 8 even when 50% of the hormone protein appears in the fast electrophoretic component after treatment in 0 1 N NaOH at 25° C for 72 minutes there is no decrease in biological potency When the hormone is treated with 0 1 N acetic acid at 25° C for 6 hours the biological activity is lost to a considerable extent with the appearance of 50% of the hormone as the fast electrophoretic component It is therefore likely that different forms of denatured hormone protein exist some of which may still possess activity depending upon the conditions of treatment and the process (I or II) of denaturation



There are so far no means of telling what physicochemical changes occur in the molecule to give rise to the active or inactive forms of the denatured protein Electrophoretic and solubility studies reveal nothing to differentiate between these two forms However it is not impossible that other physicochemical investigations might disclose certain properties which would distinguish the active denatured protein from the inactive It should be pointed out that once the protein becomes denatured it cannot revert to the native state the process denatured protein (active) → denatured protein (inactive) is also irreversible The same conditions which inactivate the native hormone also produce inactivation of the active form of the denatured protein

Action of Enzymes

In an earlier study, it has been shown that with a high ratio of enzyme/hormone (w/w) i e approximately 1 2 the hormonal activity was abolished almost completely by digestion with pepsin and trypsin at 37 C for 90 minutes No systematic investigation has since been reported concerning

Precipitation of proteins with trichloroacetic acid (TCA) has been known to cause denaturation Growth hormone in 5% aqueous TCA solution is completely insoluble at 0° C and the TCA precipitate retains growth promoting activity (Table 10) Moreover under these conditions no fast electrophoretic component appears indicating that the hormone protein remains in its native state

Table 10

EFFECT OF UREA AND TRICHLOROACETIC ACID (TCA) ON THE ACTIVITY OF GROWTH HORMONE

Treatment	*No of Rats*	*Tibia Width** (μ)
Urea† (2 0*M*)	6	249 ± 5 7§
TCA‡	23	233 ± 2 0
Untreated	77	230 ± 1 7

* A total dose of 80 μg in 4 days
† 1% solution in pH 7 0 2 0*M* urea at 25 C for 89 hours
§ Growth hormone in 1% solution was precipitated in 5% TCA solution at 0 C
‡ Mean ± standard error

Table 11

PERCENTAGE OF ELECTROPHORETIC COMPONENTS OF GROWTH HORMONE AFTER 60 SECOND REACTION* WITH CRYSTALLINE CHYMOTRYPSIN

Enzyme/Hormone†	*Electrophoretic Components‡*	
	Slow (%)	*Fast* (%)
1/50	26	74
1/100	37	63
1/300	53	47
1/2000	67	33
1/5000	100	0
0/0	100	0

* In pH 8 6 borate buffer at 25 5 C
† The ratio is on the basis of weight
‡ As estimated from the patterns obtained in experiments using an acetate buffer at pH 4 0 0 03 ionic strength

It has been known for some time that one of the initial steps in the action of proteolytic enzymes on proteins is denaturation of the substrate [40] We have recently demonstrated that when a growth hormone solution (pH 9 5) was kept in the presence of crystalline chymotrypsin at 0° C for only 30 minutes with an enzyme hormone ratio (w/w) of 1 300 the fast electrophoretic component appears with no indication according to the DNP

Table 12

QUANTITATIVE ESTIMATION OF AMINO ACIDS LIBERATED BY THE ACTION OF CARBOXYPEPTIDASE* ON SOMATOTROPIN

Amino Acid	*µm Amino Acid/9 2 mg Somatotropin*		*Residues Amino Acid Liberated/Mole*†
	Prep A	*Prep B*	
Phenylalanine	0 36	0 36	1 8
Alanine	0 10	0 10	0 5
Leucine	0 06	0 07	0 35
Serine	0 05	0 05	0 25
Threonine	0 04	0 02	0 20
Valine	0 03	0 035	0 18
Lysine	0 01	0 01	0 05
Tyrosine	0 01	0 01	0 05

* Enzyme/substrate nitrogen 1 50 incubated at pH 8 5 and 25 C for 16 hours
† The molecular weight of somatotropin is taken as 45 000

Table 13

ACTION OF CARBOXYPEPTIDASE ON SOMATOTROPIN

Experiment	*Tibia Test*‡		*Weight maintenance Test*¶			
	No of Rats	*Width of Uncalcified Cartilage of Tibia (micra)*	*No of Rats*	*Initial (g)*	*Final (g)*	*Gain (g)*
Enzymic digest A*	5	229 ± 4 8§	7	117 ± 1 2	135 ± 1 4	17 9 ± 0 6 (>0 7)**
Enzymic digest B†	4	223 ± 7 9				
Untreated hormone	77	230 ± 1 7	8	129 ± 0 2	147 ± 1 8	18 6 ± 2 3
Saline	22	148 ± 2 4	5	113 ± 1 6	112 ± 2 6	−1 0 ± 1 4

* pH 7 5-8 0 at 25 C for 16 hours ratio enzyme/substrate 1 60 two moles of phenylalanine had been removed from the protein hormone
† Further hydrolysis with carboxypeptidase of the product recovered from enzymic digest A
‡ Animal given a total dose of 80 µg in 4 days
§ Mean ± standard error
¶ Hypophysectomized male rats 40 days of age at operation were injected intraperitoneally with 0 05 mg once daily for 10 days beginning on the day of operation
** p values from Fisher s table of t

modified hormone (Digest A of Table 13) performed in order to release additional amino acids from the C terminal region did not cause any significant loss of biological activity

Partial Hydrolysis with Chymotrypsin[1] In a typical experiment growth hormone was dissolved in water to 2% solution and this was added to an equal volume of chymotrypsin solution containing 0 067 mg enzyme per cc in pH 9 5 borate buffer The solution (enzyme/hormone ratio 1/300) was incubated at 25 C in a water bath for the desired length of time The

the correlation of the degree of hydrolysis by proteolytic enzymes with changes in growth promoting activity This problem interested us for some time especially with respect to the possibility that the biological activity may not depend upon the whole molecule as is the case with ACTH In a preliminary report[41] we have demonstrated that with an enzyme/hormone ratio of 1 200 at pH 8 5 and 25° C the hormone retains its activity up to 34% of hydrolysis with chymotrypsin

Action of Carboxypeptidase[4] The uses of carboxypeptidase for the identification of C terminal amino acids in polypeptides and proteins and for the investigation of the essentiality of the C terminal group(s) for the biological activity of the molecule have now established this important technique in protein chemistry With this procedure, we have shown[4] that two residues of phenylalanine together with smaller but stoichiometrically significant amounts of alanine leucine and serine are released from the hormone protein by the action of carboxypeptidase (Fig 10, Table 12) Phenylalanine is identified as the C terminal residue of at least one and probably of both the constituent peptide chains in the hormone molecule

When the growth hormone from which two residues of phenylalanine had been removed by the enzyme was assayed for biological activity, it was found according to results of both tibia width and body weight assay that C terminal phenylalanine is not essential for the biological function of the hormone (Table 13) It is of interest to note that further digestion of the

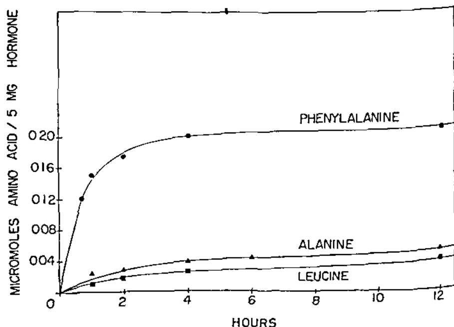


Fig 10 Rate of release of amino acids by the action of carboxypeptidase on growth hormone Enzyme/hormone = 1/50 pH 8 5 at 25° C Taken from Harris Li Condliffe and Pon[4]

Table 15

GROWTH PROMOTING ACTIVITY OF THE "CORE" FROM A PARTIAL CHYMOTRYPTIC DIGEST OF GROWTH HORMONE

Preparation	*Total Dose* (μg)	*No of Rats*	*Tibia Width* (*Mean* ± *S E*) (*micra*)
Untreated growth hormone	20	8	207 ± 5 1
	60	8	239 ± 3 6
	200	8	271 ± 4 4
"Core" of chymotryptic digest *	20	8	202 ± 3 5
	60	7	232 ± 3 1
	200	7	277 ± 5 5

* The "core" was obtained from the 5% trichloroacetic acid precipitate of a chymotryptic digest (enzyme hormone 1/300 pH 9 5 25 C 2 hrs NPN 25%)

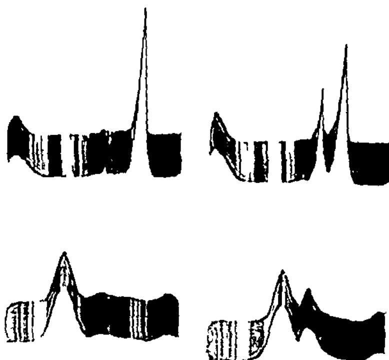


FIG 12 Electrophoretic patterns of the "core" prepared from the 5% trichloroacetic acid precipitate of a chymotryptic digest of growth hormone hydrolyzed to the extent of 23% and the "core" plus untreated growth hormone in an acetate buffer of pH 4 0 and 0 03 ionic strength 2700 seconds at a potential gradient of approx 12 volts/cm

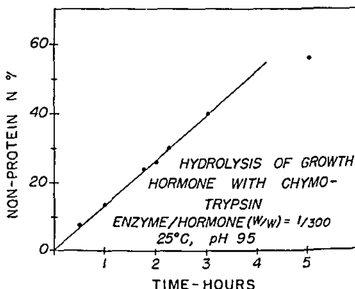


FIG 11 Rate of hydrolysis of growth hormone with chymotrypsin at 25° C Enzyme/hormone (w/w) = 1 300 in borate buffer of pH 9 5

Table 14

ACTION OF CHYMOTRYPSIN ON GROWTH HORMONE

Time of Digestion (min)*	*Non Protein Nitrogen (%)*	*Bioassay†*	
		No of Rats	*Tibia Width (micra)*
0	0	77	230 ± 1 7‡
105	24	52	234 ± 1 5
135	30	10	206 ± 2 0
300	56	13	172 ± 2 8

* Enzyme hormone (w/w) = 1 300 pH 9 5 at 25 C
† A total dose of 80 micrograms in 4 days
‡ Mean ± S E

reaction was stopped by the addition of 1 drop of glacial acetic acid The non protein nitrogen (soluble N in 5% trichloroacetic acid) was used as the index of hydrolysis It may be seen in Figure 11 that the rate of hydrolysis follows a zero order reaction up to 4 hours of digestion When the core of the digest* was assayed it was found that 24% hydrolysis does not cause loss of activity but longer digestion abolishes the growth promoting action (Table 14) An extensive multiple dose assay of the core of the 24% digest compared with the native hormone showed that there are no essential differences in biological activity between the core and untreated material (Table 15)

* The "core" is obtained by dissolving the 5% TCA precipitate in a slightly alkaline solution and dialyzing against distilled water (adjusted to pH 11) at 1 C the dialyzed solution is lyophilized

even after 46% hydrolysis The digestion was carried out in a borate buffer at pH 9 5 at 25° with an enzyme/hormone ratio of 1 300

It may also be noted from Table 17 that digestion with pepsin to an extent of 24% decreases considerably the activity of growth hormone in contrast to the results obtained with chymotrypsin and trypsin

Table 17

ACTION OF TRYPSIN AND PEPSIN ON GROWTH HORMONE

*Enzyme**	*Solvent*	*Time of Digestion at 25 C (min)*	*Non Protein Nitrogen (%)*	*Bioassay†*	
				No of Rats	*Tibia Width (μ)*
Trypsin	pH 9 5 borate buffer	40 60 120	24 30 46	10 11 6	230 ± 1 5‡ 223 ± 3 0 (0 001)§ 211 ± 1 7
Pepsin	0 01*M* HCl	120	24	12	176 ± 2 5

* Enzyme/hormone (w/w) = 1/300

† A total dose of 80 μg in 4 days untreated hormone at this dosage gives an average tibia width of 230 ± 1 7μ

‡ Mean ± standard error

§ *p* values from Fisher s table of *t*

Comments

We have summarized in this paper the present status of information about the purity of growth hormone showing that studies with new techniques in protein chemistry (zone electrophoresis adsorption chromatography counter current distribution and terminal residue analysis etc) reveal no evidence of inhomogeneity in the hormone protein It will not be until the synthetic product can be compared with the naturally occurring molecule and the two found to be identical that absolute proof will be at hand However since no procedures have yet been discovered for the synthesis of a protein and since none of the available techniques have given evidence of heterogeneity it may be assumed that the growth hormone is a homogeneous protein and that the protein is the hormone

Table 18

EMPIRICAL FORMULA FOR GROWTH HORMONE

Mol Wt 47 886

Total Residues 416

Glu[illegible]Asp CyS Ser[illegible]Thr Gly Ala Pro[illegible] Val Met (Leu + Ileu)
Phe[illegible]Tyr[illegible]LyS[illegible]Arg[illegible]Try (-NH)[illegible]

Boundary electrophoresis of the core has given no indication of the existence of a component which behaves like growth hormone In an acetate buffer of 0 03 μ at pH 4 0 electrophoresis of a solution containing the core and the native hormone gives rise to two distinct peaks with mobilities of 8 5 × 10^{-5} and 6 8 × 10^{-5} cm^2/sec/volt at 1° C The value of the slow component is identical with that of the native hormone It is of interest that the core itself does not give a complex electrophoretic pattern although its mobility is 1 3 × faster than that of the growth hormone (Fig 12)

Analyses of the N terminal residues of the core reveal that a number of new residues (threonine serine tyrosine lysine etc) appear in addition to phenylalanine and alanine If we assume that DNP phenylalanine and DNP alanine have come from the undigested growth hormone the amount of unchanged hormone in the core may be computed from the data summarized in Table 16 On this assumption it was estimated that the native hormone in the core amounts to less than 20% This is certainly not sufficient to account for the biological activity of the core From these results it is not unreasonable to assume that the activity does not depend upon the integrity of the protein and thus it may be inferred that the growth promoting activity resides in only a portion of the whole molecule

Table 16

N TERMINAL RESIDUES OF THE CORE OF A CHYMOTRYPTIC DIGEST OF GROWTH HORMONE

N terminal Residue†	Preparation of Growth Hormone*			
	Untreated		Core from Chymotryptic Digest	
	μ Mole	%‡	μ Mole	%
Ala	0 260	52 4	0 216	13 0
Phe	0 236	47 6	0 164	9 9
Leu + Ileu	0	—	0 178	10 8
Gly	0	—	0 064	3 9
Thr	0	—	0 260	15 7
Ser	0	—	0 388	23 4
Glu + Asp	0	—	0 386	23 3

* 22 5 mg were used in each case
† Values in μ mole
‡ Percentage of the total residues

Action of Trypsin [1] As has already been mentioned extensive hydrolysis of growth hormone with trypsin abolishes the biological activity It has been demonstrated recently in this laboratory that digestion with crystalline trypsin to an extent of less than 30% does not cause loss of growth promoting potency (Table 17) It is significant that some activity is still retained

form by the tissue enzymes before it exerts its hormonal function? If the *extractable* hormone does not represent the true hormone but is rather the pro-hormone it will not be surprising if future investigations find that all biologically active proteins or peptides under appropriate conditions of digestion can be partially hydrolyzed enzymatically without loss of activity We concur in what Carlson[13] pointed out as early as 1936 in the preface to the well known volume of Van Dyke s on the physiology of the pituitary gland Many of the pituitary gland products fractionated by modern biochemical methods and demonstrated to have physiological or pharmacological actions have not yet been shown to be true pituitary gland hormone —that is to be secreted into the body fluids by this gland in health or disease That various physical and chemical agents applied to the dead or dying hypophysis may develop specific chemical entities never secreted as such by the living gland is now recognized by most investigators

References

1 van Dyke H B *The Physiology and Pharmacology of the Pituitary Body* Vol II University of Chicago Press 1939 32
2 Li C H and H M Evans *Science* 99 183 (1944)
3 Li C H *Harvey Lectures* **181** (1950–1951)
4 Li C H Evans H M and M E Simpson *J Biol Chem* **159** 353 (1945)
5 Wilhelmi A E Fishman J B and J A Russell *J Biol Chem* **176** 735 (1948)
6 Li C H *J Biol Chem* (in press)
7 Greenspan F S Li C H Simpson M E and H M Evans *Endocrinology* **45** 455 (1949)
8 Geschwind I I and C H Li see elsewhere in this volume
9 Li C H *Amino Acids and Proteins* Ed D M Greenberg Springfield Ill Charles C Thomas Publisher 1951 487
10 Li C H and M Moskowitz *J Biol Chem* **178** 203 (1949)
11 Li C H and K O Pedersen *J Biol Chem* **201** 595 (1953)
12 Li C H and H Papkoff (unpublished results)
13 Fønss Bech P and Li C H *J Biol Chem* **207** 175 (1954)
14 Li C H and Clauser H (unpublished results)
15 Northrop J H *J Gen Physiol* **13** 739 (1930)
16 Butler J A V *J Gen Physiol* **24** 189 (1940)
17 Cohn E J *Ann Rev Biochem* 4 136 (1935)
18 Clauser H and C H Li *J Am Chem Soc* **76** 4337 (1954)
19 Zittle C A *Advances in Enzymol* **14** 319 (1953)
20 Moore S and W H Stein *Ann Rev Biochem* **21** 521 (1952)
21 Craig L C and D Craig *Techniques of Organic Chem* 3 171 (1950)
22 Harfenist E J and L C Craig *J Am Chem Soc* **74** 3083 (1953)
23 Harfenist E J *J Am Chem Soc* **75** 5528 (1953)
24 Cole R D and C H Li Fed Proc **13** 193 (1954)
25 Pierce J G *Biochem J* **57** 16 (1954)
26 Sanger F *Biochem J* **39** 507 (1945)
27 Edman P *Acta Chem Scand* 4 283 (1950)
28 Li C H and L Ash *J Biol Chem* **203** 419 (1953)

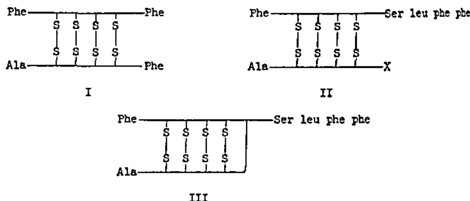


FIG 13 Possible structure of growth hormone

With the acceptance of this conclusion chemical investigations into the fine structure of the hormone protein are more significant and the empirical formula for growth hormone may be written as in Table 18 From the analytical data one of the structures shown in Figure 13 may represent the true structure of the hormone molecule

Until further investigations are carried out on the C terminal residues with chemical techniques it is difficult to decide which of the proposed structures is the correct one Scheme II is based on the hypothesis that one of the amino acid residues represented by x is resistant to the action of carboxypeptidase However there is justification for favoring Scheme III If the structure were either I or II oxidation of the —S—S— bridges would give rise to two fragments one with N terminal phenylalanine and the other with N terminal alanine However in preliminary studies on the oxidation of growth hormone with performic acid zone electophoresis reveals that the product of oxidation remains a single component Furthermore there is no reason to exclude the possibility of the formation of a peptide bond by the γ–COOH group of aspartic or glutamic acid the assumption upon which this structure is based

The fact that the biological activity resides only in a portion of the whole protein molecule is not new The earlier experiments with thyroglobulin and the partial peptic digest of ACTH are well known The non essentiality of the C terminal residue(s) for the biological function of an active protein appears to be common This is found to be true not only for growth hormone but also for insulin tobacco mosaic virus and lysozyme

From the results of these enzymatic studies one may wonder about the *actual* nature of protein or peptide hormones as they occur in the body as physiologically active catalysts *Is the hormone we extract from the gland* or tissue the *true* hormone? Might it be that the extractable hormone is the pro hormone? Is it possible that the hormone molecule after entering into the circulation through parental administration is converted into another

difficult it might be to separate two proteins which resembled each other a little bit

There is also the consideration that one can run a paper electrophoresis with certain proteins with an agent such as bromophenol blue attached to the protein The color will move with the protein without changing its mobility at all Thus it is possible that the protein could carry a small molecule along with it We have had the experience also in the pituitary field of corticotropin being isolated as a pure protein of molecular weight 20 000 which protein later was referred to as pure ACTH We now know it was just a slightly contaminated protein and the active material was apparently just stringing along with the inert protein This is a difficult field and I think it is too soon to arrive at a final conclusion

Dr Wilhelmi s very interesting paper creates more problems for those of us interested in isolating active growth fractions We are now faced with the possibility of having a number of species specific hormones We should be reluctant as long as we can to accept the conclusion that there are different hormones because it will make the identity of growth hormone so much more difficult It is true in the case of the human use of corticotropin that when crude preparations were being used some patients actually failed to respond This seems to be well documented and it happened quite a few times Patients failed to respond to the intramuscular or subcutaneous injections of crude corticotropin and did respond later to highly purified corticotropin I am still slightly hopeful that we might be facing some similar situation here It may be that in the evolution of new species the tropic end organs such as thyroid adrenal cortex etc didn t change as much as the soma and now they require new pituitary hormones to be stimulated I hope this is not the case

I should like to present data on two experiments done with an impure growth hormone preparation which nevertheless I am very fond of In Figure 1 is shown the result of a 16 transfer counter current distribution of a growth hormone preparation prepared by the glacial acetic extraction method It demonstrates that during 16 transfers a large portion of material (note that the vertical scale is shortened) stayed in the initial aqueous phase and did not move out into the organic phase at all Now of the material that did move away from the zero tube we have plotted the aqueous phase against the organic phase and have found that there was a constantly changing distribution ratio This could mean that we had any number of materials In bio assaying the material from various points on the distribution curve we found activity in the material that didn t move at all and in the material from the higher tube numbers There was activity in between as well We tried to extend this distribution a little more to see if we could get some further pattern The study was done with the help of Dr Paul Bell at the Stamford Laboratories of the American Cyanamid Company We took only the butanol extractable material and left behind the

29 Levy A L and C H Li (unpublished data)
30 Popenoe E A Pierce J G du Vigneaud V and H B van Dyke *Proc Soc Exp Biol Med* **81** 506 (1952)
31 Franklin A L Li C H and M S Dunn *J Biol Chem* **169** 515 (1947)
32 Moore S and W H Stein *J Biol Chem* **178** 53 (1949)
33 Moore S and W H Stein *J Biol Chem* **192** 663 (1951)
34 Levy A L *Nature* **174** 126 (1954)
35 Li C H Levy A L and D Chung (unpublished results)
36 Goodwin T W and R A Morton *Biochem J* **40** 628 (1946)
37 Geschwind I I (unpublished) cf Levy A L Geschwind I I and C H Li *J Biol Chem* (in press)
38 Li C H and H Papkoff *J Biol Chem* **204** 391 (1953)
39 Li C H and H M Evans *The Hormones* **1** 631 (1948)
40 Linderstrom Lang K *Proteins and Enzymes* Calif Stanford Univ Press 1952
41 Condliffe P and C H Li *Fed Proc* **11** 198 (1952)
42 Harris J I Li C H Condliffe P G and N G Pon *J Biol Chem* **209** 133 (1954)
43 Carlson A J in van Dyke H B *The Physiology and Pharmacology of the Pituitary Body* University of Chicago Press 1937 p vii

DISCUSSION

Bioassay, Preparation and Physicochemical Properties of Growth Hormone

Designated Discussion

MAURICE S RABEN (New England Center Hospital) I find it rather reassuring to listen to Dr Li particularly to hear that he has arrived at the point of submitting a structure for growth hormone I have not found the molecule so readily understandable and I have not found its behavior so uniform as to be anywhere near reaching that point

I can just mention a few things one runs into in handling growth hormone On the one hand it can be extracted with hot glacial acetic acid It can be treated with an alkyl aryl detergent and heated in this without loss of activity This of course is one of the most denaturing procedures one can use The hormone can stay in a counter current distribution machine for 10 days with organic solvents and retain its activity On the other hand if it is just touched with pepsin at room temperature its activity vanishes In contrast to that Dr Li says that it can resist chymotrypsin digestion Some time ago Dr Wilhelmi told me and I have confirmed this that active growth hormone can be extracted from pituitary glands which have been allowed to thoroughly rot We tried it as long as the people in the laboratory would allow Activity is still present when the tissue is obviously putrefying Thus I am not at all sure what to make of all this The matter of actually proving purity of proteins is a very difficult one We have seen recently that it has taken 30 years to separate glucagon from insulin There is no resemblance at all in those two proteins and one can readily appreciate how

rial that didn t run at all and in the material with the most rapidly moving peak The activity in the latter was perhaps a little stronger than that in the somewhat slower moving peak There was even some activity in the smeared material The small peak on the left was the only section in which we definitely found no activity I don t know how to interpret all this on the basis of one independent substance behaving in an independent manner

Designated Discussion

STANLEY ELLIS (University of California) First I would like to say that I certainly admire the presentations and continued work of Dr Wilhelmi and Dr Li on the chemistry and preparation of growth hormone They have contributed more than anyone else to this problem One of the real hopes of the investigators who study the biological effects of growth hormone is to have a preparation which is a pure substance at least by all our present physical and chemical criteria With the advent of newer methods for determining purity what is said one day may have to be retracted the next At the Institute of Experimental Biology we have been concerned with an attempt to prepare for biological purposes a growth hormone which is a pure one judged by classical procedures We have been using for our methods that of Drs Li and Pedersen a modification of this which might briefly be called the Li and Pedersen modification and also the procedure of Dr Wilhelmi This work has been going on for a period of two or three years and in the course of that time we have introduced some modifications which have decreased the contamination with TSH and ICSH The conclusion which I would like to present after this extended period of work is that growth hormone as prepared by any of the above mentioned methods has a contaminant when judged by our electrophoretic studies i e free electrophoresis This contaminant appears at pH 9 6 and with low ionic strength and it amounts to about 10% It is the most difficult thing to remove from growth hormone preparations It is not just an electrophoretic anomaly for it is also evident when one uses the constant solubility test of Northrop It might be said perhaps that growth hormone has been obtained pure in one or two instances However I must say that there will be great difficulty in reproducing such efforts to give the biologist a physically and chemically pure preparation

The second comment is along the lines of growth hormone stability In the past year or so we have been engaged in determining the stability of growth hormone in terms of electrophoretic transformations and biological activity We have studied the behavior of the hormone following its exposure to molar acetic acid glacial acetic acid various temperatures and to the alkaline extreme of normal sodium hydroxide In respect to these two pH extremes we thoroughly agree with Dr Li in his conclusion that growth hormone exposed to acetic acid loses its growth activity with time

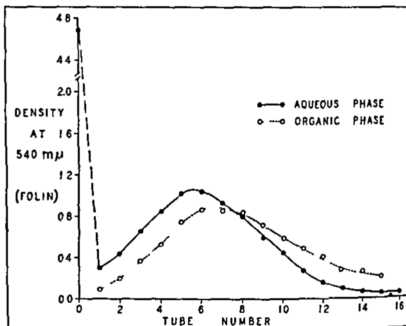


Fig 1

material which did not move at all In other words we extracted the growth hormone with the organic solvent and used only that fraction This was then run for 679 transfers the fractions being taken off the end of the 220 tube automatic machine In this instance we used 750 mg of the extracted material and charged it in 8 tubes The solvent system was 05 molar sodium chloride and 0 1 molar acetic acid against secondary butanol Based on the nitrogen determination we found one small peak as noted on the left of Figure 2 a rather smear of material noted in the center of the plot and two distinct major peaks seen on the right When we assayed the material for growth hormone activity we found growth activity in the mate

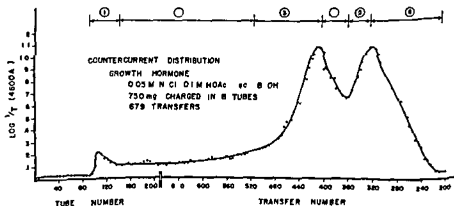


Fig 2

the paper I am referring to the fat mobilization effect in fasting mice to the muscle glycogen maintaining effect in hypophysectomized rats and to the growth promoting activity as measured by body weight growth as well as to the growth promoting activity in hypophysectomized mice They all *indicate that the core material has essentially the same activity as our* general protein

STANLEY ELLIS No Dr Engel, we haven t as yet tested the alkali treated hormone for the activities you mentioned

S J FOLLEY (University of Reading England) I was very interested in Dr Wilhelmi s results on the species specificity of the various growth hormones Last summer the University of Liverpool organized a symposium on comparative endocrinology and during part of this meeting I occupied the chair There was a good deal of discussion on the question as to whether or not hormones themselves have changed during evolution or was it merely the uses to which they were put I wonder whether Dr Wilhelmi s finding that the molecule of the fish growth hormone is smaller than the mammalian substance could be cited as evidence that the molecular structures of the hormones themselves have undergone evolutionary changes

LAURANCE W KINSELL (Highland Alameda County Hospital) In Dr Russell s presentation earlier this morning it was apparent that there are a few difficulties inherent in the various assay methods which have been used for growth hormone In our experience that has been true to an extent that we would question whether or not there is an assay available which can be called at the present time a quantitative or specific procedure for growth hormone in any real sense of the phrase Should this be at all true then I think it must be recalled and kept constantly in mind that purity of growth hormone, in a chemical sense is entirely dependent upon the soundness of the assay methods which are available This applies not only to growth hormone but to the other pituitary factors It was reported today that such things as antibiotics could make the increase in epiphyseal width an unreliable assay for growth hormone I suspect that anything which can increase appetite in hypophysectomized animals under study will make the assay *equally unreliable In this respect even tiny amounts of ACTH which* certainly can increase appetite in hypophysectomized humans might be a real interfering factor

ERIC REID Referring to Dr Engel s question I would like to add that diabetogenic activity of alkali treated growth hormone in cats parallels its growth promoting activity in rats

KARL E PASCHKIS (Jefferson Medical College) I should like to comment for a minute on Dr Geschwind s paper and on the factors in the tibia test

We have been unable, as was Dr Li to detect any loss of activity when the hormone was kept in sodium hydroxide for 3 hours at room temperature However, in the pH region from 5 to about 11, growth hormone can be permitted to stand for at least 24 hours without undergoing any electrophoretic transformations or showing any loss of activity At about pH 11 5 electrophoretic change begins to appear With that one exception in the *more or less neutral pH range we have confirmed quite well that growth* activity can be lost in glacial acetic acid and that with prolonged treatments in sodium hydroxide for instance 6 hours growth activity is also lost

General Discussion

C N H LONG (Chairman) It is quite evident there is a good deal of room for difference of opinion concerning the properties of growth hormone

ERIC REID (Royal Cancer Hospital England) In connection with Dr Li's paper I might just mention some observations made in collaboration with Dr Wilhelmi as to whether the so called purified protein might contain some active constituent We were encouraged in this search partly because some years ago I had shown that the terminal amino groups on alanine and phenylalanine were not necessary for the activity of the protein preparation Our approach was to subject the dry preparation to fractional extraction with organic solvents the material having been set up as a column The solvents, passed through the column were initially rich in acetone in which the hormone is insoluble Increasing the amounts of an acetic constituent such as acetic acid eventually led to the elution of some material which we examined in various ways In some instances we were able to get active material off the column in low yield In no instance however, did this material have an activity in rats greater than that of the original growth hormone This was true even when we employed aluminum phosphate to delay absorption in case some small molecule was absorbed so fast we couldn't spot its activity In no instance could we see any obvious chemical difference between the fractions taken off the column and the original material So far then we have no evidence against the view that the protein is the hormone and we certainly could not show any active constituents in this protein

FRANK L ENGEL (Duke University) Will Dr Li tell us whether he has tested for any other biological activity in his hormone treated various ways particularly the material treated in alkali? It would be interesting also to know whether Dr Ellis has measured other activities of growth hormone I am referring to the fat mobilizing ketogenic and diabetogenic activities

CHOH HAO LI (University of California) We have measured two or three additional biological properties of the core material which I mentioned in

Part II

Effects of Growth Hormone on Certain Structures

Chairman

Frederick L. Hisaw
The Biological Laboratories
Harvard University
Cambridge Massachusetts

which condition the response to growth hormone It was mentioned that thyroxin given to the hypophysectomized animal produced an increase in the epiphyseal width while testosterone in the dose employed, did not On the other hand, testosterone given to the hypophysectomized animal will induce nitrogen retention which is a measure of protein anabolism while thyroxin given to the same animal will not Now, that reveals a rather intriguing difference of response to these hormones, if one uses the different parameters of growth Is it a matter of sensitivity of the different tests or to flip the coin around is it a matter of dosage? Are we dealing with an inherent difference in the response of a specific tissue, i e a difference between the localized tibia response and the overall metabolic response in the total animal?

ABRAHAM WHITE (Yeshiva University) Taking for the moment Dr Li's presentation I wish to ask him two questions regarding the core material which is obtained from chymotrypsin digestion Firstly, in view of the appearance of new end groups does he have any data regarding the number of molecular species present in this core Secondly does he have any data regarding the average molecular weight of the individuals in this core material?

CHOH HAO LI I was aware of this as a possible question before I started to talk about the core material today and I am not surprised that it was Dr White who asked it I must say that the core material is very complex It probably consists of many different components In respect to the molecular weight we have no definite data but I suspect that it must be smaller than that of the original protein

STANLEY ELLIS I would like to ask Dr Li whether he has found any bioactivity in the hormone following chymotrypsin digestion and in the supernatant following precipitation with 5 per cent trichloroacetic acid (TCA)?

CHOH HAO LI Before I answer Dr Ellis question I should mention that urea at pH 7 did not affect the activity The 5 per cent TCA precipitate of the original protein hormone did not show loss of activity Now, the supernatant from 5 per cent TCA precipitation most likely contains very little activity but I cannot give you a black and white answer to that question

6

Growth Hormone (Somatotropin) and the Glands of the Digestive System[*]

Burton L Baker and Gerald D Abrams[†]
Department of Anatomy University of Michigan Medical School Ann Arbor Michigan

Twenty five years have elapsed since the classic publication by Philip Smith[1] entitled Hypophysectomy and a replacement therapy in the rat made the technique of hypophysectomy available as a routine method for investigation of pituitary physiology One of the striking sequels which he observed was cessation in growth of the body In searching for an explana tion of this effect it is indeed surprising that investigators have paid so little attention to the structural and functional condition of the digestive tract after pituitary ablation Possibly this is because biochemists have emphasized the changes which occur in intermediary metabolism Thus Samuels Reinecke and Bauman demonstrated that deamination is in creased and anabolism of protein reduced after hypophysectomy They observed that the differences in nitrogen balance between hypophysecto mized and non hypophysectomized rats could not be accounted for by dif ferences in rate of digestion and absorption as indicated by the excretion of fecal nitrogen Such evidence relegates the digestive system to a place of secondary importance

However the available information indicates clearly that some aspects of the supportive influence exerted by the hypophysis on the digestive sys tem are the result of direct hormonal action either by hormones of the hypophysis itself or by those of other endocrine glands controlled by it

This investigation was supported (in part) by a research grant (A 131 (C2)) from the National Institutes of Health Public Health Service and by grants from the University of Michigan Memorial Phoenix Project (#57) and The Upjohn Company
† We extend our appreciation to Mrs Delores McGinty Mrs Norma Reid and Miss Mary Masten for their technical assistance

Defective absorption after hypophysectomy might result in part from incomplete digestion of food This possibility focuses attention on the competency of serous cells to produce digestive enzymes Some of the cells (serous or zymogenic) which are known to contribute enzymes to the gastro-intestinal contents or which on the basis of their cytology might be expected to do so are summarized in Table 1 During the past three years we have been investigating the influence of hormones on certain of these enzyme producing cells The results are still fragmentary In this report we will describe the effects of hypophysectomy on the structure and function of some of these cells (Table 1) and will present evidence concerning the role of somatotropin and other hormones in restoring the deficiencies which arise

Gastric Chief Cells

Hypophysectomy in rats results in an involution of the chief cells which is accompanied by depletion of pepsinogen granules and cytoplasmic ribonucleic acid[1] (Fig 1a b) This effect occurs promptly and is clearly evident within 7 days Concurrently a reduction occurs in the concentration of and total peptic activity in the gastric juice which is secreted during a 6 hour period after pyloric ligation The capacity to secrete pepsin is depressed 3 days after hypophysectomy but reaches its lowest point at 7 days (Table 2)

Table 2

EFFECT OF HYPOPHYSECTOMY ON SECRETION OF GASTRIC JUICE AND ON ITS PEPTIC ACTIVITY*

Treatment	*Days after Hyp*	*No of Rats*	*Mean Vol (ml)*	*Mean PU × 10 /ml†*	*Mean Total PU × 10 ⁴/Sample*
Hyp	3	8	4 3 ± 0 6‡	80 0 ± 8 9	325 ± 34
Con		8	7 3 ± 0 6	131 7 ± 6 4	949 ± 74
			P§ = < 0 01	P = < 0 001	P = < 0 001
Hyp	7	9	4 6 ± 0 4	45 8 ± 10 3	199 ± 38
Con		10	10 5 ± 0 8	128 3 ± 6 0	1344 ± 112
			P = < 0 001	P = < 0 001	P = < 0 001
Hyp	105	15	5 4 ± 0 4	50 5 ± 5 4	262 ± 28
Con	128	4	11 5 ± 3 5	99 7 ± 7 1	1200 ± 426
			P = < 0 01	P = < 0 001	P = < 0 001

Hyp = hypophysectomy Con = control

* Baker and Abrams *Am J Physiol* 177 409 (1954)

† PU = hemoglobin proteolytic units as measured by mEq of tyrosine released from hemoglobin by the pepsin of gastric juice during 1 minute of digestion at 35 5 C

‡ Standard error

§ Student Fisher *t* test significance of differences

Clarification of the hormonal means by which the hypophysis modifies the cytology and secretory capacity of chief cells is aided by an analysis of

Hypophysectomy has the following effects (a) The weight of the intestine in pigeons is reduced to a greater extent than can be accounted for by reduced food intake[3] (b) In cats the weight of the mucosa of the stomach small intestine colon and rectum, is reduced and is histologically atrophic[4] (c) Similar observations are reported by Friedman for the rat with the small intestine being more severely affected than the stomach He further claims that the parietal cells are reduced in number by 50% (d) The pH of gastric juice is increased[6] (e) The amount of secretin in the intestinal mucosa[7] and amylase in the pancreas[8] is reduced (f) The absorption of glucose is depressed[9] apparently because of hypoactivity of the thyroid[10] Dysfunction of the digestive tract is implicated further by the observation of Shaw and Greep[11] that the general condition of the body and apparently the synthesis of protein more nearly approximate normal if hypophysectomized rats are fed purified diets rather than commercial laboratory chow In these studies the purified diets may have been digested more easily by the functionally deficient alimentary tract of the hypophysectomized animals

Alleviation of some of these deficiencies has been attempted by hormonal therapy of hypophysectomized rats The weight of the intestine and pancreas in pigeons is restored most effectively by lactogen and thyrotropin[3] Somatotropin induces a marked increase in weight of the stomach and intestine in hypophysectomized rats[1] The reduction in secretin content of the intestine is prevented by the administration of crude anterior pituitary extracts somatotropin or corticotropin Thyroxine is ineffective[13] The amylase activity of the pancreas in hypophysectomized rats is stimulated by thyroxine[8] and the rate of absorption of glucose is restored to normal by the same hormone[14]

Table 1

SOME SEROUS OR SEROMUCOUS GLANDS ASSOCIATED WITH THE DIGESTIVE TRACT

- I Oral and Pharyngeal
  - A Anterior lingual buccal labial etc
  - B Salivary
    - 1 Parotid*
    - 2 Submandibular*
    - 3 Sublingual*
- II Esophageal
  - A Cardiac
- III Gastric
  - A Fundic (chief cells)*
  - B Cardiac pyloric
- IV Intestinal
  - A Crypts of Lieberkuhn (cells of Paneth)
  - B Associated Glands
    - 1 Brunner s
    - 2 Pancreas*

* These glands are discussed in the text

the effects of removal of other endocrine glands which are under pituitary control Gonadectomy does not alter the cytology of chief cells or their capacity to secrete pepsin The effect of thyroidectomy is similar except that the total peptic activity is reduced because of lessened production of gastric juice [16 17] Adrenalectomy results in some involution of the chief cells and suppression of pepsin secretion [19] These changes are not comparable with those which ensue after hypophysectomy but become so if thyroid ectomy and gonadectomy are combined in the same animal with adrenal ectomy [17] These observations suggest that the anterior hypophysis regulates the chief cells mainly through the adrenal cortex

Table 3

EFFECT OF SOMATOTROPIN ON THE SECRETION AND PEPTIC ACTIVITY OF GASTRIC JUICE

Treat ment	No of Rats	Mean Vol (ml)	Mean PU* × 10 /ml	Mean Total PU × 10 , Sample
1 Control	8	13 1 ± 0 99†	76 3 ± 13 0	947 3 ± 123 0
2 Hypophy sectomy	7	5 3 ± 0 44	43 3 ± 7 2	226 3 ± 40 8
3 Hyp Som ‡	5	8 5 ± 1 71	46 8 ± 10 3	349 1 ± 66 3
Group 1 vs 2		P = < 001	> 05	< 001
Group 2 vs 3		P = > 05	> 05	> 05

* PU = hemoglobin proteolytic units or mEq tyrosine released
† Standard error
‡ Somatotropin 1 mg daily for 7 days beginning 9–13 days after hypophysectomy

The activity of chief cells in hypophysectomized animals must be restored to normal with replacement therapy before the role of the anterior hy pophysis can be assessed with finality This is of particular importance be cause certain studies indicate that neurohypophyseal hormones modify gas tric secretion [0 21] However this conclusion is denied by Gross Ingram and Fugo[2] as a result of carefully controlled studies To date therapy with thyroxine * cortisone acetate hydrocortisone somatotropin and force feeding separately and in various combinations have been tried Adaptation to force feeding by stomach tube was accomplished over a period of 7 days by gradually increasing the amount of food administered Then hypophy sectomy was performed and the feeding continued for another 7 days All of the hormonal treatments were carried out for 7 days having initiated therapy one or 7–12 days after hypophysectomy Thyroxine (3µg /day)

* We wish to thank Squibb and Company for the L thyroxine Merck and Company for cortisone acetate and hydrocortisone and The Armour Laboratories for somato tropin (Lots 285–128 and 285–183)



FIG 1 Chief cells of fundic stomach Hotchkiss and methylene blue stain (A) Non hypophysectomized control The staining of the cytoplasm is due to the presence of ribonucleic acid The apical cytoplasm is vacuolated because the pepsinogen granules were not preserved (B) Hypophysectomized 17 days previously The chief cells (arrow) are involuted (C) Hypophysectomized for 17 days previously and treated with somatotropin 1 mg daily for last 7 days Ribonucleic acid is increased greatly over that in (B)

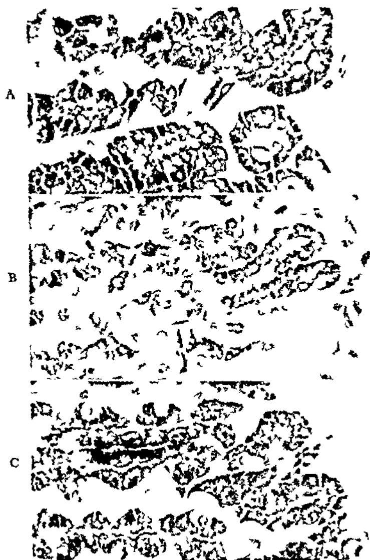


Fig 2 Chief cells of fundic stomach Toluidine blue and eosin stain (A) Non hypophysectomized control (B) Hypophysectomized 8 days previously (C) Hypophysectomized 8 days previously and treated with 1 mg of hydrocortisone daily during the last 7 days The involution of the chief cells is retarded as shown by comparison with (B)

does not change the cytology of the chief cells Somatotropin and force feeding increase somewhat the size of the cells and amount of ribonucleic acid as indicated by basophilic staining (Fig 1) However, the result falls far short of achieving the normal picture Similarly, somatotropin when injected at a 1 mg daily dose for 7 days, induces an insignificant change in pepsin secretion (Table 3) The best cytological effect was obtained with hydrocortisone when therapy (1 mg /day) was begun the day after hypophysectomy In this case the involution of chief cells which occurred during the subsequent 7 days was not as severe as that in non treated hypophysectomized rats (Fig 2) Similarly replacement therapy with 0 5 mg of cortisone acetate daily elicits a significant rise in the secretory capacity of chief cells (Table 4)

Table 4

EFFECT OF CORTISONE ACETATE ON THE SECRETION AND PEPTIC ACTIVITY OF GASTRIC JUICE

Treatment	No of Rats	Mean Vol (ml)	Mean PU* × 10 ⁴/ml	Mean Total PU × 10 ⁴/Sample
1 Control	6	13 7 ± 1 32†	98 8 ± 13 3	1294 ± 129 0
2 Hypophysectomy	7	5 3 ± 0 44	61 9 ± 5 0	328 ± 37 9
3 Hyp + CA ‡	6	8 7 ± 0 53	98 3 ± 3 7	848 1 ± 53 4
Group 2 vs 3		P = < 001	< 001	< 001

* PU = hemoglobin proteolytic units or mEq tyrosine released
† Standard error
‡ Cortisone acetate 0 5 mg daily for 7 days beginning 9–13 days after hypophysectomy

It would be premature at this time to draw a final conclusion concerning the route of pituitary control over the chief cells Many more replacement experiments must be carried out in which dosages and duration of hormonal treatment are explored intensively At the moment it appears that the chief cells are affected by many factors Thus food intake[18] and somatotropin modify the content of ribonucleic acid this substance being of great importance in the synthesis of protein enzymes Of the agents tested adrenocortical hormones are the most effective in stimulating the secretion of pepsin by chief cells of the hypophysectomized animal although they have not restored the normal cytological picture These results indicate that a major route of pituitary control is through the adrenal cortex This hypothesis is supported by extensive studies in man [3] which reveal an increased secretion of pepsin and excretion of uropepsin during adrenocortical therapy That the adrenal cortex is not the only route of pituitary control

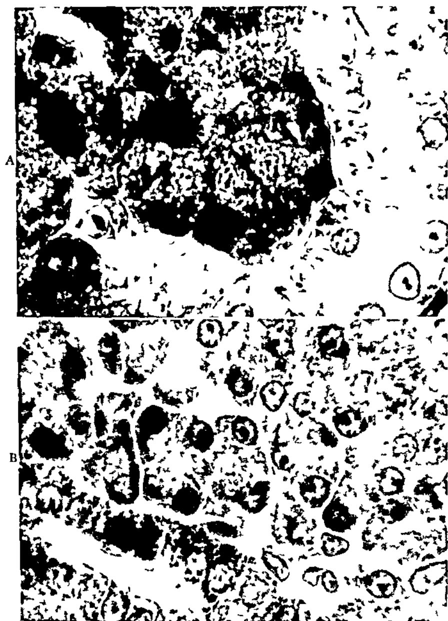


FIG 3 Acinus and a duct from the parotid gland Zenker formol fixation Altman Masson stain (A) Non hypophysectomized control The acinar cells (center) are engorged with zymogenic granules compressing the nucleus against the base of the cell (B) Hypophysectomized 35 days previously Zymogenic granules are depleted from the acinar epithelium and the nuclei are no longer compressed The ductular epithelium (lower left) is lower and still contains many mitochondria

is proven by the failure of adrenalectomy to result in the severe structural and functional changes in the chief cells which occur following hypophysectomy

Salivary Glands

Parotid Of all salivary glands the parotid is affected most severely following hypophysectomy The gland weight is reduced markedly when compared with that of non operated control animals (Table 5) and the acini

Table 5

WEIGHT OF THE SUBMANDIBULAR AND PAROTID GLANDS 35 DAYS AFTER HYPOPHYSECTOMY

Group	*No of Rats*	*Submandibular*		*Parotid*	
		Gland (mg)	*G W /B W **	*Gland (mg)*	*G W /B W*
Control	10	171 ± 20†	0 95 ± 0 12	238 ± 57	1 32 ± 0 3
Hypophysectomy	10	92 ± 3	0 71 ± 0 07	74 ± 17	0 57 ± 0 14
		P = < 001	> 05	< 001	< 05

* Gland Weight (mg) / Body Weight (g)
† Standard deviation

involute with an accompanying and striking loss of zymogenic granules (Fig 3) Administration of corticotropin to non hypophysectomized rats has a similar effect [4] The basophilia of the acinar cells is reduced but much still remains Recent studies in this laboratory by Mr Gerald Klegman demonstrate that the parotid gland of the rat contains a high concentration of amylase activity which is depressed significantly by hypophysectomy In attempting to restore the lost weight of the gland after pituitary ablation the administration of thyroxine (3μg /day for 7 days) proves ineffective Treatment with somatotropin brings about an increase in the absolute weight the gland weight/body weight ratio not being altered significantly (Table 6) * The correlation of the data on this increase in weight with that on the histological picture is not completed but it can be stated that the increase in weight is not due to the accumulation of fat in the stroma of the gland Cortisone acetate induces some increase in weight which is not statistically

* The means and standard deviations of the body weights for the rats treated with somatotropin (Tables 6 and 9) were as follows At the time of hypophysectomy non hypophysectomized controls 188 ± 7 g hypophysectomized 184 ± 5 hypophysectomized somatotropin treated 187 ± 5 At the time of initiation of hormone treatment 201 ± 14 161 ± 7 and 165 ± 11 respectively At autopsy 208 ± 11 159 ± 10 and 199 ± 17 g respectively

an enzyme The functional significance of the acinar epithelium is in doubt According to Grad and Leblond [5] it produces mucus because the cells stain with PAS (periodic acid leucofuchsin) after fixation in bichromate formol After fixation of our material in formalin alcohol acetic acid only sparsely scattered PAS staining granules appear which certainly cannot account for all of the intracellular secretion since the apical cytoplasm remains extensively vacuolated Zenker formol fails to preserve the substances contained by these vacuoles We agree with others [6] that the acinar epithelium cannot be eliminated as a possible source of enzymes

The absolute weight of the submandibular gland is significantly less in hypophysectomized rats as compared with their non operated controls but when considered in relation to body weight this difference is not significant (Table 5) The acinar epithelial cells are only slightly smaller and contain a moderately reduced amount of intracellular secretion Ribonucleic acid is retained in great quantity However the height of the epithelium of the duct system is strikingly lower and the serous granules disappear from the intralobular tubules The submandibular gland of the mouse is affected similarly by hypophysectomy [7]

Therapy with somatotropin increases the weight of the gland in hypophysectomized rats in proportion to the gain in body weight (Table 6) The granulation of serous tubules is restored partially Since thyroxine[28] and androgens [5 7] have been shown to elicit this granulation in hypophysectomized rats the thyrotropic activity in our growth hormone preparations may have been responsible However cortisone acetate (0 5 mg daily for 7 days) increases the absolute weight of the gland (Table 7) which seems doubly significant because the body weight of these animals continues to fall off The latter effect is partially responsible for the significant increase which occurred in the gland weight/body weight ratio Thus the adrenal cortex may have a more important influence on the submaxillary gland than has been realized heretofore

Sublingual The primary function of the sublingual gland is the secretion of mucus rather than enzymes Thirty five days after hypophysectomy its weight is less than that of the glands in the non-operated control rats (Table 8) However this change is not out of proportion to the lesser body weight The histological effects are minor and are featured by a greater concentration in the intracellular mucus which results in more intense staining These changes are of minor significance when compared with those occurring in serous cells which are known to produce enzymes

Pancreas

The weight of the pancreas is reduced significantly following hypophysectomy when considered on an absolute basis or in relation to body weight (Table 8) [28 9] Thirty five days after the operation the acini are smaller and contain fewer zymogenic granules although they are con

Table 6

EFFECT OF SOMATOTROPIN ON THE MEAN WEIGHT OF SUBMANDIBULAR AND PAROTID GLANDS AFTER HYPOPHYSECTOMY

Treatment	No of Rats	Submandibular		Parotid	
		Gland (mg)	G W /B W *	Gland (mg)	G W /B W
1 Control	12	173 ± 23†	0 83 ± 0 11	175 ± 37	0 85 ± 0 21
2 Hypophysectomy	10	96 ± 11	0 60 ± 0 04	90 ± 23	0 57 ± 0 14
3 Hyp + Som ‡	10	127 ± 19	0 63 ± 0 08	133 ± 48	0 68 ± 0 23
Group 1 vs 2	P = < 001		< 001	< 001	< 001
Group 2 vs 3	P = < 001		> 05	< 05	> 05

* Gland Weight (mg) / Body Weight (g)
† Standard deviation
‡ Somatotropin 1 mg daily for 7 days beginning 10–14 days after hypophysectomy

Table 7

EFFECT OF CORTISONE ACETATE ON THE WEIGHT OF SUBMANDIBULAR AND PAROTID GLANDS AFTER HYPOPHYSECTOMY

Treatment	No of Rats	Submandibular		Parotid	
		Gland (mg)	G W /B W *	Gland (mg)	G W /B W
1 Control	4	176 ± 19†	0 92 + 0 09	185 ± 22	0 97 ± 0 10
2 Hypophysectomy	5	83 ± 4	0 56 ± 0 03	92 ± 12	0 63 ± 0 10
3 Hyp + CA ‡	4	115 ± 8	0 82 ± 0 07	112 ± 27	0 81 ± 0 16
Group 1 vs 2	P = < 001		< 001	< 001	< 01
Group 2 vs 3	P = < 001		< 01	> 05	> 05

* Gland Weight (mg) / Body Weight (g)
† Standard deviation
‡ Cortisone acetate 0 5 mg daily for 7 days beginning 14 days after hypophysectomy

significant (Table 7) At present the hormonal pathway for control of this gland is not clear but somatotropin may play an important role

Submandibular As inferred from their cytology one and possibly two portions of the submandibular gland possess the capacity to secrete enzymes Certain segments of the intralobular ducts are lined by an epithelium which contains serous granules These probably represent precursors of

pituitary ablation fails to increase the weight of the pancreas However cortisone acetate given under similar conditions at a dose of 0 5 mg /day increases the absolute weight and gland to body weight ratio (Table 10)

Table 10

EFFECT OF CORTISONE ACETATE ON THE MEAN WEIGHT OF SUBLINGUAL GLAND AND PANCREAS AFTER HYPOPHYSECTOMY

Treatment	*No of Rats*	*Sublingual*		*Pancreas*	
		Gland (mg)	*G W B W **	*Gland (mg)*	*G W /B W*
1 Control	4	38 ± 7†	0 2 ± 0 04	893 ± 110	4 7 ± 0 6
2 Hypophysectomy	5	26 ± 5	0 18 ± 0 03	369 ± 67	2 5 ± 0 4
3 Hyp + CA ‡	4	35 ± 6	0 25 ± 0 04	516 ± 59	3 7 ± 0 4
Group 1 vs 2	P =	< 05	> 05	< 001	< 01
Group 2 vs 3	P =	< 05	< 01	< 02	< 01

* Gland Weight (mg)
Body Weight (g)
† Standard deviation
‡ Cortisone acetate 0 5 mg daily for 7 days beginning 14 days after hypophysectomy

Discussion

This review represents only a beginning in the analysis of the hormonal regulation of enzyme producing cells but demonstrates that herein lies a fertile field for future investigation Several generalizations have become evident which may serve as guides to future work and which are pertinent to the objectives of this symposium

First it is erroneous to regard all exocrine glands as being controlled to an equal degree by the anterior hypophysis Rather these glands must be considered with respect to the type of secretion which each produces On this basis the exocrine glands associated with the digestive tract fall into 3 general categories (a) mucous (b) serous or zymogenic and (c) mixed the latter appearing to produce a mixture of mucous and serous secretions The cytology of the acini of the mucus secreting type as exemplified by the sublingual gland undergoes only minor modification after hypophysectomy The acini of the submandibular gland whose role in mucous and serous secretion is poorly understood also are affected to only a moderate degree At the opposite extreme glands or their parts which are composed primarily of serous or zymogenic cells e g chief cells in fundic glands the parotid gland pancreas and serous tubules of the submandibular gland are affected much more significantly by the loss of pituitary hormones In these cases involving the elaboration of enzymes the structural features of this involution are those which characterize cells whose synthetic capacity is suppressed

stantly present After hypophysectomy, the faint basal chromophilia of the epithelium (fixed in formalin alcohol acetic acid and stained with methylene blue) is reduced and amylase activity is depressed [8] Miss Nancy Thoms has demonstrated recently in this laboratory that the protease activity also shows a striking reduction

Table 8

WEIGHT OF THE SUBLINGUAL GLAND AND PANCREAS 35 DAYS AFTER HYPOPHYSECTOMY

Group	No of Rats	Sublingual		Pancreas	
		Gland (mg)	G W /B W *	Gland (mg)	G W /B W
Control	10	45 ± 6†	0 248 ± 0 04	804 ± 86	4 47 ± 0 5
Hypophy sectomy	10	31 ± 4	0 239 ± 0 03	328 ± 75	2 53 ± 0 6
		P = < 01	> 05	< 001	< 001

* Gland Weight (mg) / Body Weight (g)
† Standard deviation

Somatotropin therapy for 7 days fails to induce a weight gain in the pancreas of hypophysectomized rats in spite of a marked gain in body weight (Table 9) Kinash et al [30] observed a significant increase in the absolute weight when treatment was continued for 21–28 days This rise fell short of restoring the normal body weight Nishikawara et al [8] found thyroxine to elicit a considerable recovery in amylase activity In our experiments treatment with L thyroxine (3µg /day for 7 days), beginning 7 days after

Table 9

EFFECT OF SOMATOTROPIN ON THE MEAN WEIGHT OF THE SUBLINGUAL GLAND AND PANCREAS AFTER HYPOPHYSECTOMY

Treatment	No of Rats	Sublingual		Pancreas	
		Gland (mg)	G W /B W *	Gland (mg)	G W /B W
1 Control	12	37 ± 7†	0 18 ± 0 03	802 ± 113	3 8 ± 0 4
2 Hypophy sectomy	10	32 ± 4	0 20 ± 0 02	455 ± 68	2 9 ± 0 4
3 Hyp Som ‡	10	30 ± 5	0 16 ± 0 03	480 ± 113	2 4 ± 0 6
Group 1 vs 2		P = < 05	> 05	< 001	< 01
Group 2 vs 3		P = > 05	< 05	> 05	> 05

* Gland Weight (mg) / Body Weight (g)
† Standard deviation
‡ Somatotropin 1 mg daily for 7 days beginning 10–14 days after hypophysectomy

studies presented the data and observations are concerned primarily with the reserve of stored enzyme rather than with rates of secretion Glands of the digestive system undoubtedly contain far more enzyme at any one time than is needed by the body This concept is compatible with the large reserve of tissue known to be possessed by many other organs If one of the primary defects in hypophysectomized animals is a change in protein metabolism which reduces the animal s requirement for amino acids it follows that a smaller quantity of digested and absorbed food will suffice Hence the deficient secretory capacity of zymogenic cells in a hypophysec tomized animal may still be adequate for his depressed level of living This agrees in essence with the conclusion derived by Samuels et al [2] from bio chemical studies which holds that the deficiency in absorption of food is of secondary importance to the metabolic disturbances which occur after hypophysectomy Nevertheless the deficiencies induced by pituitary abla tion in the zymogenic cells are so severe that it seems premature to eliminate them as having a small primary role in the chain of events leading to cessa tion of growth

References

1 Smith P E *Am J Anat* 45 205 (1930)
2 Samuels L T Reinecke R M and K L Bauman *Endocrinology* 33 87 (1943)
3 Schooley J P Riddle O and R W Bates *Am J Anat* 69 123 (1941)
4 Haeger K Jacobsohn D and G Kahlson *Acta Physiol Scand Suppl 111* 30 161 (1953)
5 Friedman M H F *J Natl Cancer Inst* 13 1035 (1953)
6 Crafts R C and B S Walter *Endocrinology* 40 395 (1947)
7 Dorchester J E C and R E Haist *J Physiol* 118 188 (1952)
8 Nishikawara M Barrett J Maykut M Sprague L and R E Haist *Fed Proc* 13 105 (1954)
9 Russell J A *Am J Physiol* 121 755 (1938)
10 Althausen T L in *Essays in Biology in Honor of Herbert M Evans* Berkeley Univ Calif Press 1943
11 Shaw J H and R O Greep *Endocrinology* 44 520 (1949)
12 Simpson M E Evans H M and C H Li *Growth* 13 151 (1949)
13 Dorchester J E C and R E Haist *J Physiol* 119 266 (1953)
14 Russell J A *Proc Soc Exp Biol Med* 37 569 (1938)
15 Baker B L and G D Abrams *Am J Physiol* 177 409 (1954)
16 Sun D C H Shay H Siplet H and M Gruenstein *Gastroenterology* 27 189 (1954)
17 Abrams G D and B L Baker *Gastroenterology* (in press)
18 Baker B L and R M Bridgman *Am J Anat* 94 363 (1954)
19 Tuerkischer E and E Wertheimer *J Endocrinology* 4 143 (1945)
20 Cutting W C Dodds E C Noble R L and P C Williams *Proc Roy Soc (London) B* 123 27 (1937)
21 Cutting W C Dodds E C Noble R L and P C Williams *Proc Roy Soc (London) B* 123 49 (1937)
22 Gross E G Ingram W R and N W Fugo *Am J Digest Diseases* 9 234 (1942)

Second insofar as biochemical studies have been carried out on enzym producing tissues the cytological involution which follows hypophysectomy correlates rather well with the reduction in content or secretion of enzymes This holds for the secretion of pepsin by chief cells, the content of amylase in the parotid and the content of amylase and protease in the pancreas

Third, it is impossible to draw conclusions at the present time concerning the nature of hormonal control over the various zymogenic cells Only a beginning has been made in the essential experiments involving replacement therapy These are difficult and require thorough exploration of dose and time response relationships Our attempts at replacement therapy after hypophysectomy thus far have fallen considerably short of complete restoration of a normal cytological or functional state in the zymogenic cells Hence deductions made from available information must be viewed with caution

Treatment with somatotropin increases the weight of the parotid and submandibular glands in hypophysectomized rats as a part of the general growth response of the body It remains to be demonstrated that this constitutes a partial functional recovery Although we found somatotropin to be without effect on the pancreas Kinash et al[30] reported it to cause an increase in weight Nevertheless these investigators pointed out that some unknown factor is still required for full recovery Because of the presence of contaminating principles in growth hormone preparations more studies must be carried out to eliminate the possible involvement of other endocrin secretions in the induction of these responses Somatotropin exerted some effect on gastric chief cells but failed to restore the normal complement of pepsinogen granules as well as the capacity to secrete pepsin

Viewing all of the evidence available it appears that an important route over which the anterior hypophysis modifies the activity of at least some zymogenic cells is through the adrenal cortex with some effect being mediated through thyroid gland Sufficient knowledge has been acquired to show that such a generalization cannot be applied strictly to all of the zymogenic glands and that clarification of the hormonal regulation of each gland represents a special endocrine problem Probably there is no one hormone which when given to a hypophysectomized animal can restore an involuted zymogenic cell to a normal state of activity Without doubt a normal cytological state and optimal function are the outcome of the interplay on the cell of many hormones and nutritional factors as is true of most metabolic processes

Finally what is the significance of the depressed enzyme secreting capacity of zymogenic cells to the growth stasis which follows the hypophysectomy? Several facts are relevant One is faced with the over riding observation that somatotropin can restore a nearly normal rate of growth as indicated by gain in body weight (a 20% increase within 7 days of treatment) *without* full restoration of structure or enzyme content of zymogenic cells In the

Effect of Somatotropic Hormone (STH) Upon Inflammation

Hans Selye

Institut de Medecine et de Chirurgie experimentales Universite de Montreal
Montreal Canada

Introduction

The voluminous literature concerning the effect of the pituitary adreno cortical system upon inflammation and infection has been surveyed in a series of monographs [1 3 4] In view of this and of the limitations in time I shall restrict my discussion here to those studies on the action of STH upon inflammation about which I can speak from personal experience

We became interested in this topic as a result of observations which revealed that it is possible to *produce inflammatory changes* (e g myocarditis arteritis arthritis) in animals by treatment with a crude lyophilized anterior pituitary preparation (LAP) [6 7] as well as with desoxycorticosterone acetate (DCA) [8] Thus it has been demonstrated that true inflammatory changes can be produced by hormonal agents [7] under suitable experimental conditions

Subsequent work demonstrated that these same hormone preparations are even more effective in enhancing inflammatory responses to local irritants (e g frostbite formalin) that is they augment the inflammatory potential of tissues [9 10] The prophlogistic effect of LAP and of the most highly purified STH preparations now available is roughly parallel to their growth promoting action hence it is highly probable (though still not certain) that this activity of pituitary extracts is due to their STH content [11 1]

Interestingly after adrenalectomy both LAP and STH completely lose their ability to produce spontaneous sclerotic and inflammatory changes in the kidney heart and vessels and yet they retain their ability to stimulate inflammatory responses to direct tissue injury for instance in joints or sub

23 Gray S J Ramsey C Reifenstein R W and J A Benson Jr *Gastroenterology* **25** 156 (1953)
24 Baker B L *Recent Progr Hormone Research* **7** 331 (1952)
25 Grad B and C P Leblond *Endocrinology* **45** 250 (1949)
26 Stormont D L in *Special Cytology* Ed E V Cowdry **1** 153 (193?)
27 Lacassagne A and A Chamorro *C R Soc Biol* **134** 223 (1940)
28 Koster S *Pfluger's Arch* **224** 212 (1930)
29 Bryans F E Kinash B Ashworth M A and R E Haist *Diabetes* **1** 358 (1952)
30 Kinash B MacDougall I Evans M A Bryans F E and R E Haist *Diabetes* **2** 112 (1953)

the *prophlogistic corticoids* (P C) stimulate the proliferative ability and reactivity of connective tissue they enhance the inflammatory potential Thus they help to put up a strong barricade of connective tissue through which the body is protected against further invasion by the pathogenic stressor agent

However under ordinary conditions ACTH stimulates the adrenal much more effectively to secrete *antiphlogistic corticoids* (A C) These inhibit the ability of the body to put up granulomatous barricades in the path of the invader in fact they tend to cause involution of connective tissue with a pronounced depression of the inflammatory potential Thus they open the way to the spreading of infection

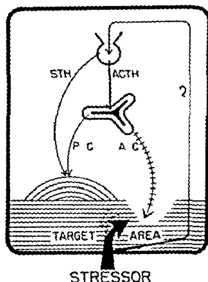


Fig 1

As far as we know ACTH always stimulates the adrenal to produce the various corticoids in the same proportion and always with a great predominance of A Cs However the *somatotropic hormone* (STH) of the pituitary also increases the inflammatory potential of connective tissue somewhat as the P Cs do hence it sensitizes the target area to the actions of the latter

It is possible that the hypophysis also secretes some special corticotropin which induces the adrenal to elaborate predominantly P Cs indeed STH itself may possess such effects but this has not yet been proved In any event if ACTH were the only corticotropin the actions of the corticoids produced under its influence can be vastly different depending upon conditioning factors (such as STH) which specifically sensitize the target area for one or the other type of corticoid action Actually conditioning factors could even alter the response to ACTH of the adrenal cortex itself so that its cells would produce more A Cs or P Cs Thus during stress one or the other type of effect can predominate

The fundamental reaction pattern to topical stressors is inflammation to systemic stressors the G A S Various combinations of these two basic responses constitute the essence of most diseases [1]

cutaneous tissue exposed to irritants [2 3 13 14] It was thought probable therefore, that the effects of STH upon the kidney heart and the cardiovascular system are mediated through the adrenals while their prophlogistic actions upon other tissues are largely direct [1] However, even this non adrenal mediated effect of STH can be greatly enhanced by simultaneous treatment with DCA as shown by experiments on adrenalectomized rats [16]

On the other hand certain earlier findings had suggested that the adrenal can also exert *antiphlogistic actions* For instance, during the alarm reaction when intense systemic stress augments ACTH and gluco-corticoid secretion certain inflammatory responses (e g, the anaphylactoid reaction of the rat to egg white) are inhibited This antiphlogistic effect of stress also appeared to be dependent in some way upon the adrenal, since it was abolished by suprarenalectomy [17] The importance of this inhibitory action was only revealed however after the discovery[18] that ACTH and cortisone exert marked antirheumatic effects As a result of the many experiments which this fundamental observation on man inspired throughout the world, it may now be taken as definitely established that while STH and DCA like compounds are prophlogistic ACTH and cortisone like hormones are antiphlogistic Thus the inflammatory potential of the organism largely depends upon *the ratio between the pro and anti inflammatory hormones*

From a clinical point of view it is particularly significant that the antiphlogistic hormones tend to decrease *resistance to infection* This may be largely due to the fact that they prevent the formation of a granulomatous barricade around foci of micro organisms (thus facilitating the spread of the latter) although to some extent the inhibition of serologic defense reactions by these substances may also be involved in this phenomenon [19] On the other hand STH and the prophlogistic corticoids tend to increase resistance to infections presumably because their action upon these same defense mechanisms is inverse [19 20 21]

For the sake of simplicity let us disregard meanwhile the still somewhat obscure role played by the renal pressor system and summarize our views on the humoral regulation of inflammatory responses in the form of a diagram

As shown in Figure 1 the *stressor* acts upon the *target* (the body or some part of it) directly (thick arrow) and indirectly through the pituitary and adrenal.

Through some *unknown pathway* (labelled by a question mark) a stimulus travels from the directly injured target area to the *anterior pituitary* It notifies the latter that a condition of stress exists and thus induces it to discharge ACTH

It is quite possible that this first mediator of hormonal defense is not always the same In some instances it may be an adrenaline discharge in others a liberation of histamine like toxic tissue metabolites a nervous impulse or even a sudden deficiency in some vitally important body constituent (such as glucose or an enzyme)

ACTH stimulates the *adrenal cortex* to discharge corticoids Some of these

the consequent development of the blood supply) can be blocked by con current STH treatment [30]

It may be appropriate at this time to survey the basic technical *prerequisites of such experiments* since up to now we did not have enough experience in this field to do so and several otherwise excellently planned published studies failed to yield positive results because of purely technical flaws in their design

(1) *In any experiment concerning the importance of a well balanced hormonal tension it is essential to have four experimental groups treated as follows*

I *Untreated* controls

II An *antiphlogistic* hormone (e g ACTH cortisol cortisone) given under experimental conditions and at a dose level permitting at least one of its typical effects to become clearly manifest (e g inhibition of inflammation sensitization to infection growth inhibition and catabolism atrophy of lymphatic organs)

III A *prophlogistic* hormone (STH DCA) given under the same conditions at a dose level at which one of its typical effects is clearly manifest (e g stimulation of inflammatory granuloma and exudate formation growth and anabolism enlargement of the lymphatic organs)

IV *Simultaneous treatment with the pro and antiphlogistic* hormones at dose levels which are so balanced against each other that a complete neutralization (balanced hormonal tension) results and the indicator of hormonal action (e g body weight inflammatory potential) is indistinguishable from the corresponding target in the untreated controls (Group I)

It is actually Group IV which determines the dose levels to be used in Groups II and III respectively For instance in an experiment in which we wish to examine the effect of a hormonal tension between cortisol and STH upon a certain infection in the rat we may decide arbitrarily to employ cortisol at the daily dose level of 1 mg and use a slowing of the growth rate as a well-established indicator of this hormone s activity Under these conditions the rats of Group II should grow at a distinctly subnormal rate as compared to those of Group I while the rats in Group IV must receive as much STH as is necessary to maintain a normal growth rate despite the cortisol treatment Unless this condition of perfect balance with regard to a known antagonistic function is fulfilled it can hardly be expected that the experiment could reveal an antagonism between these hormones with respect to a hitherto unexplored function (for instance a special kind of infection)

It may be added that if the antiphlogistic hormone is given at a dose level at which it is manifestly effective in respect to one of its characteristic actions (Group II) and the prophlogistic hormone is given at a dose level at which it nullifies this effect when given conjointly with the former (Group IV) then—at least in all cases which we have so far examined—the effect of the prophlogistic hormone given alone (Group III) was always quite evident and opposite in direction to that of the antiphlogistic hormone It is for this reason that we may regard the response in Group IV as an indicator determining the dosages to be used in the other groups

(2) *If an antiphlogistic and a prophlogistic hormone are completely balanced against each other in intact animals under basic conditions subsequent exposure to a stressor usually shifts the balance in favor of the antiphlogistic hormone*

Thus for instance if cortisol and STH are perfectly balanced with regard to

Relationship between the Antiphlogistic and the Anticatabolic Actions of STH

Intense catabolism produced by severe systemic stress is almost invariably accompanied by a diminution of the inflammatory potential, hence it is noteworthy that STH can diminish or even abolish loss of body weight normally caused by various stressors This was clearly shown for instance in rats exposed to *X rays* Even the loss of body weight elicited by an *inflammatory irritant* (turpentine) can be thus counteracted [3]

In these particular experiments we did not verify whether the inhibition of catabolism by STH is actually accompanied by a restoration of the normal inflammatory potential However this was found to be so in animals in which STH was employed to counteract the catabolic actions of *glucocorticoids* Simultaneous treatment with these two opposing types of hormones leads not to a simple neutralization of both in every respect but rather to a complicated interplay of antagonisms and synergisms this has been referred to as hormonal tension

To use a mechanical analogy the complete relaxation of both agonistic and antagonistic muscles may place an extremity in exactly the same position as the balanced contraction of both these opposing muscle groups yet the resistance of the limb to external forces will be quite different We believe that such hormonal antagonisms are important in determining resistance to many diseases and particularly to those accompanied by inflammation Hence we shall discuss this topic at some length now

The Phenomenon of "Hormonal Tension"

The growth inhibition normally caused by ACTH (as well as several of its metabolic actions) can be compensated by simultaneous treatment with STH [4] In certain experiments on rats it was shown furthermore that among the effects of cortisone the involution of the adrenal cortex and of the thymus as well as the antiphlogistic effect (judged by the *topical irritation arthritis test*) are counteracted by simultaneous STH treatment Curiously even those organs which normally undergo an absolute or relative increase in size as a result of either cortisone or STH treatment (e g the heart kidney liver) are maintained in an essentially normal weight range if both hormones are given conjointly [6]

The inflammation produced by croton oil with the *granuloma pouch* technique [16] the healing of experimentally produced *gastric ulcers*, [7] of *burns*-[8] and of the *noma like erosions* which can be produced by mechanical trauma in the oral cavity of the rat [9] furnished further proof that under the most varied conditions the inhibition of granuloma formation by cortisone or cortisol can be counteracted by STH Even the growth depressing effect which cortisol exerts upon certain *transplantable malignant tumors* (presumably due to interference with the inflammatory stroma reaction and

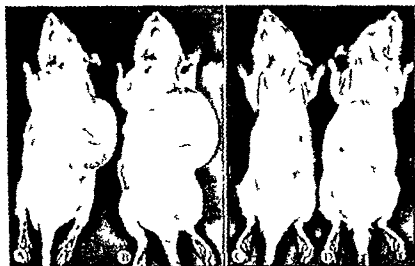


FIG 2 Effect of STH and cortisol upon the development of a granuloma pouch In all these animals a granuloma pouch was produced with croton oil A Otherwise not treated B STH treated C Topically treated with cortisol (see two white crystal deposits which form longitudinal streaks along both lateral borders of pouch) Almost complete disappearance of the pouch as a result of the antiphlogistic hormone D Treated with STH (as B) and cortisol (as C) The hormone deposits are again visible Under the influence of STH the antiphlogistic effect of cortisol is partially abolished (After Selye[18])

Dependence of Certain STH Effects upon the Adrenals

The production of myocarditis or periarteritis by DCA can be prevented in the rat by hypophysectomy [3, 33] This suggests that the pituitary is necessary to make the tissues sensitive for this prophlogistic action of the mineralo-corticoid substance Conversely—as we have said in our introductory remarks—the prophlogistic actions of DCA are augmented by simultaneous STH treatment

Recently we have performed a large series of experiments on intact and adrenalectomized rats in which these interrelationships have been studied using the granuloma pouch technique This is the test object currently in use at our Institute for the study of the cortisol neutralizing effect of various steroids For such assays adrenalectomized rats (usually females weighing 160 g) are maintained solely on Purina Fox Chow and tap water *without salt supplements* Under these conditions rats treated exclusively with cortisol (400 µg /day) tend to lose weight and their response to croton oil in the granuloma pouch (25 ml of air + 0 5 ml of 1% croton oil in corn oil) is subnormal they also tend to show pronounced atrophy of the thymus lymph nodes and spleen On the other hand if such animals also receive certain cortisol neutralizing steroids (e g DCA acetoxypregnenolone

their effect on growth subsequent treatment of the experimental animals with drugs microbes special diets etc automatically shifts the balance so that the cortisol effects usually predominate although treatment with both hormones is continued at the same dose level

This is presumably due to the circumstance that systemic stress conditions most target organs *for* the actions of the antiphlogistic catabolic hormones (ACTH A C s) *against* the effects of the anabolic prophlogistic substances (*STH M C s) To avoid errors resulting from this type of conditioning* (which may change in the course of an experiment as the cumulative effect of constant stress rises) it is important to verify that the hormonal tension remains balanced Thus if we decide to keep the daily dose of the prophlogistic hormone constant that of the antiphlogistic principle must be gradually reduced during systemic stress situations of increasing severity The degree and rate of this reduction are easily gauged by the results in Group IV if we take care throughout the course of the experiment always to give as much antiphlogistic hormone as is necessary to maintain the selected indicator of activity (e g growth) at the level of the untreated controls

It may happen of course that despite such neutralization of the two opposite hormone effects as regards one indicator, a perfect balance of their action will not be obtained as regards another We have frequently observed for instance that under certain circumstances the body weight of rats maintained on definitely growth inhibiting doses of cortisol could be perfectly restored to normal by simultaneous STH treatment and yet they exhibited thymus atrophy or a depression in their phlogistic potential (that is their ability to respond with inflammation to topical injury) An elucidation of the mechanism (e g selective conditioning of various targets) through which such selective dissociations in hormonal antagonism can occur would be of major importance It is impossible to appraise these points however unless two known hormonal antagonists are demonstrated to have abolished each other s actions at least in one respect Without ascertaining this point we cannot exclude the possibility that one of the two antagonists was given at dose levels (and under circumstances of conditioning) which are so excessive that none of the opposing hormonal actions could have become manifest [6]

With this in mind it is hardly unexpected that for instance in an experimental series in which mice were treated simultaneously with STH and cortisone at dose levels which did not even permit a prevention by the STH of the cortisone induced weight loss there was also no demonstrable antagonism as regards the effects of these substances upon a variety of infections [31]

To some extent STH can neutralize even the effects of an antiphlogistic corticoid topically applied to a focus of inflammation Thus cortisol injected directly into the wall of a granuloma pouch inhibits both the formation of granuloma and of exudate At the same time at different levels of hormone activity there exists an inverse proportion between the development of an inflammatory barrier around the irritant (croton oil) and the tendency of adjacent tissues to undergo necrosis These local actions of cortisol (the inhibition of granuloma and exudate formation as well as the facilitation of tissue necrosis) are effectively counteracted by systemic treatment with STH[18] as illustrated by the adjacent photographs

desoxocortisone or progesterone) all these effects of cortisol are abolished or diminished Since the anti-cortisol activity of the various steroids proved to be roughly parallel to their mineralo-corticoid potency a relationship between these two pharmacologic actions was suspected[31] It is especially noteworthy that in this test 10 μg of DCA suffices to inhibit 400 μg of cortisol

Under ordinary conditions STH resembles mineralo-corticoids in its ability to inhibit all the above mentioned gluco-corticoid effects[1,5] hence we anticipated that it would also do so in this test It was noted however that *in adrenalectomized rats maintained exclusively on cortisol without salt supplements* STH—even in very high doses (2 × 1 mg /day subcutaneously)—caused little or no growth and did not inhibit the thymolysis the splenic involution or the antiphlogistic effect (as judged by the granuloma pouch test) of cortisol as it does in intact rats Apparently either an intake of excess salt (e g substitution of tap water by 1% NaCl as a drinking fluid) or treatment with a mineralo-corticoid hormone (e g DCA) is essential for the prophlogistic lympholytic and general growth stimulating effects of STH Salt supplements are only slightly effective in this respect but as little as 100 μg /day of DCA suffices to restore the normal growth stimulating and prophlogistic effects of STH in the adrenalectomized rat (whether it is maintained on a threshold amount of cortisol or not) These observations furnish further support for the concept that a close synergistic relationship exists between STH and the M C s

Dependence of Certain STH Effects upon the Thyroid

In the rat thyroidectomy offers considerable protection against the toxic overdosage manifestations normally caused by STH or DCA In particular there is a great diminution in the ability of these substances (used singly or in combination) to produce nephrosclerosis and myocarditis On the other hand thyroidectomy does not prevent the ability of STH to stimulate growth and the thymotropic action of combined treatment with STH and DCA is particularly pronounced in the absence of thyroid tissue[3]

In a more recent study Salgado[36] showed that in the intact rat STH induces hypertension nephrosclerosis polyuria myocarditis renal cardiac and adrenal enlargement Under these same conditions a partially purified preparation of thyrotropic hormone (TTH) induced only slight inflammatory changes and enlargement of the kidney and heart polyuria and hypertension In thyroparathyroidectomized rats all these effects of TTH are abolished except for minimal renal and cardiac enlargement STH induces periarteritis nodosa and arthritis adrenal cardiac and renal enlargement and slight lesions in the kidney The other manifestations seen in the intact animal are absent The data do not exclude the possibility that the slight effect of the TTH preparation was due to contamination with STH

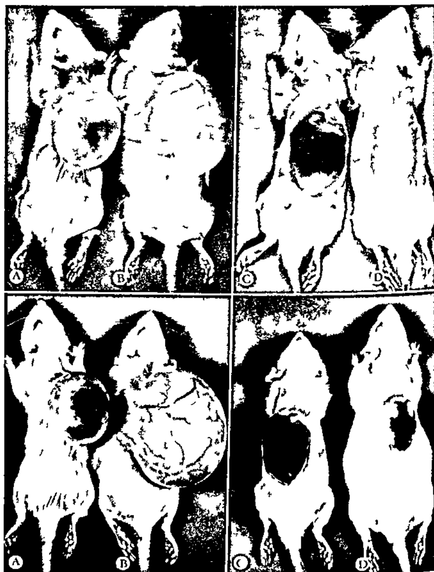


Fig 3 Effect of STH and cortisol upon resistance of granuloma pouch to otherwise necrotizing doses of croton oil Same rats are shown in Fig 2 *Top row* 24 hours *bottom row* 72 hours after injection of 0 5 ml of 100% croton oil into the already formed pouch C Necrosis is almost immediate and extensive in animals receiving cortisol alone B It is completely absent in those treated with STH alone in which the pouch merely enlarges and becomes better vascularized as a result of further irritation A Not hormone treated control and D Animals treated with cortisol and STH show necrosis in the roof of the pouch but this develops more slowly and is of lesser extent than in rats receiving cortisol alone (After Selye[16])

local growth stimulating action at the site of injection somewhat as cortisol exerts a topical inhibitory influence

Using an electrophoretically pure STH preparation (kindly supplied by Professor C H Li of Berkeley California) it has been possible to demonstrate in rats that at the site of subcutaneous or intracutaneous administration it intensely stimulates the proliferation of fibroblasts In the treated region there develops a granuloma consisting of large fibroblasts rich in rather basophilic cytoplasm and surrounded by collagenous fibers In the capillaries and small arterioles and venules there ensues a marked hyperplasia of the endothelial cells which sometimes assume an actually cylindric form All these actions are particularly pronounced if STH is injected into the vicinity of a joint It was therefore tentatively concluded that this hormone may act directly and not through the intermediary of some other organ[39]

It must be kept in mind however that none of these changes are absolutely specific for STH and although in many control experiments with other proteins we have seen no comparable degree of connective tissue stimulation the effect could nevertheless be due to some non specific irritating property of the STH protein Yet other experiments also appear to support the view of a direct effect of STH upon connective tissue Stebbins and Stoerk[40] found that saturation of cotton plugs with cortisone inhibits their ability to stimulate inflammatory granuloma formation upon subsequent subcutaneous implantation in the rat while impregnation with STH exerts an opposite effect When both hormones are simultaneously applied they mutually antagonize each other in agreement with the principle of hormonal balance Essentially similar experiments have been performed in intact hypophysectomized and adrenalectomized animals with the result that in every case when both hormones were incorporated into the same cotton plug the local depressive action of cortisone was reversed by STH Control extracts prepared by the same method from a variety of tissues other than the pituitary body proved devoid of such activity Similarly the local application of a highly purified ACTH preparation (corticotropin B) devoid of growth hormone activity did not influence the amount of granulation tissue Since adrenalectomized and hypophysectomized rats form normal amounts of granulation tissue it appears that neither the adrenal gland nor the pituitary body is essential for the response to the foreign body However excessive amounts of cortisone or STH have profound influence upon granulation tissue formation The authors incidentally also mention preliminary experiments which indicate that local administration of STH results in acceleration of wound healing

It is also interesting in this connection that the atrophy produced by topical application of cortisol to the skin under suitable experimental conditions[41] as well as the stunting of regional growth induced by local treatment of the paw nose ear etc in the rat[44] are only insignificantly in

Resistance to STH

Species Resistance The fact that currently available STH preparations cause little if any anabolism in man has been discussed at some length at this Meeting by others Let us merely point out that the guinea pig (whether intact or hypophysectomized) is also singularly resistant to the growth promoting action of STH even when preparations are used which at comparable dose levels produce marked growth in the rat It may be useful to have an experimental animal which resembles man in its apparent irresponsiveness to the growth promoting effect of STH Since several actions of STH are largely dependent upon conditioning factors (e g diet simultaneous treatment with steroids) the guinea pig may furnish us with a welcome experimental test object in which to explore such possibilities by large scale experiments [37]

Acquired Resistance Several investigators have noticed that if rats are chronically treated with high doses of the now available STH preparations an irresponsiveness tends to develop after a few weeks or months The question arose whether this insensitivity is due to the anti hormone formation to some metabolic process accelerating the actual destruction of STH or to a loss of responsiveness within the target organs perhaps resulting from the accelerated growth itself

To analyze this problem experiments were performed on undernourished rats which received so little food that they could not grow despite continuous STH treatment It was noted that after ten weeks—when the normally fed controls which became giants but ceased to grow—an immediate excessive growth spurt could be obtained in the undernourished STH treated animals by merely giving them food *ad lib* In one such experiment partially starved rats which weighed only 85 g despite ten weeks of intense STH treatment suddenly grew to 245 g within the next three weeks during which food was allowed *ad lib* During the same time interval the body weight of the similarly STH treated control rats (which were well fed throughout the experiment) rose only from 330 to 340 g [38] Evidently here it was not the duration of pretreatment but the ability of STH to actually manifest its growth effect that was the decisive factor in the development of resistance

As we shall see below giant rats which became resistant to any further growth stimulation after long continued administration of STH lose body weight rapidly if treatment be discontinued This also shows that the resistance develops only with regard to further growth while the hormone is still capable of maintaining an excessive body weight

Topical Actions of STH Preparations

In view of the manifold factors which influence the responsiveness of connective tissue to STH it is of interest whether this hormone can exert a

(1) *Relationship between the antiphlogistic and anticatabolic actions of STH* The hormone (STH) counteracts the loss of body weight normally produced by X rays inflammatory irritants or gluco corticoids In general this effect runs parallel with the inhibition by STH of the suppression of inflammation that tends to accompany catabolic reactions However it has not yet been demonstrated that this parallelism is obligatory in all cases

(2) *The phenomenon of hormonal tension* When STH is given simultaneously with ACTH or gluco corticoids it antagonizes some but not all of the effects caused by the latter hormones The complex interactions between these humoral principles which result when they are given in amounts balanced as regards catabolism have been described as hormonal tension The inhibitory effects of antipholgistic corticoids upon inflammation wound healing and even the development of certain transplantable malignant tumors can be counteracted by concurrent treatment with STH The technical prerequisites of such hormonal tension experiments have been described in detail

(3) *Dependence of certain STH effects upon the adrenals* Some effects of STH-overdosage (particularly the production of nephrosclerosis) are dependent upon the integrity of the adrenals others (e g anabolic effects stimulation of inflammation in response to injury) are exhibited even after adrenalectomy The possible corticotropic actions of STH have been surveyed on the basis of these observations

In adrenalectomized rats maintained exclusively on cortisol and not receiving an excess of NaCl STH is quite ineffective in stimulating inflammation or growth Some mineralo-corticoid effect is indispensable for the manifestation of the normal and prophlogistic effects of STH

(4) *Dependence of certain STH effects upon the thyroid* Thyroidectomy offers considerable protection against certain toxic overdosage manifestations of STH (nephrosclerosis myocarditis) while it aggravates others (mesenteric periarteritis nodosa)

(5) *Resistance to STH* The guinea pig like man appears to be singularly resistant to STH and hence may serve as a test object for the study of factors involved in species resistance to the available STH preparations

In the event of long continued treatment all available STH preparations cease to stimulate growth beyond a certain maximal level in the rat This resistance does not appear to be due to antihormone formation or to an actual destruction of the hormone If growth is prevented by partial starvation during prolonged STH treatment there ensues an immediate growth spurt as soon as food is allowed *ad lib* This is true even at a time when normally fed control rats (which are allowed to grow) have already developed resistance

Hypophysectomized rats chronically treated with STH until they become giants maintain their excessive size for a long time if STH injections are continued however they exhibit a sudden intense catabolism upon with

hibited by systemic treatment with STH in doses sufficiently high to produce gigantism Thus the disproportion between the organs stunted by topical hydrocortisol treatment and the rest of the body becomes even more marked *under such conditions The antiphlogistic effect of cortisol topically applied* to a granuloma pouch is also only slightly (though quite definitely) inhibited by systemic STH administration [16]

"Withdrawal Effects" after Discontinuation of STH Treatment

Many of the actions of STH proved to be reversible following discontinuation of treatment Thus in a large series of hypophysectomized Sprague Dawley female rats—all of which weighed 130 g on the average at the beginning of the experiment—treatment with ascending doses, up to 10 mg of STH (Armour) per day raised the body weight to an average of 450 g and caused a marked partially ankylosing polyarthritis in all animals by the 132nd day *At that time treatment was interrupted* in half of the animals and within one month these lost an average of 100 grams while those in which STH injections were continued maintained their body weight at essentially the same level The polyarthritis also improved only in the rats in which STH treatment was discontinued Of course the weight of the untreated hypophysectomized rats did not change during the whole period of observation

It is noteworthy that here resistance was acquired as regards the further growth effect of the STH once a plateau was reached but the hormone was still manifestly active since withdrawal of it led to a sudden loss of weight [43]

It may be mentioned incidentally that despite the high doses used STH failed to cause any detectable change in the weight of the adrenals of the hypophysectomized animals as compared with untreated controls This is all the more noteworthy since in intact animals the same STH preparation does cause adrenocortical stimulation and when given in conjunction with ACTH it augments the corticotropic effect of the latter as described previously [44] it also increased the formation of PAS positive granules by methylandrostenediol (MAD) in suitably sensitized intact rats [45]

The influence of these withdrawal effects upon inflammation has not yet been studied but it is noteworthy in connection with the primary object of our presentation that the increase in PAS positive adrenal granules induced by STH in MAD treated animals is associated with (though perhaps not the cause of) an aggravation of the sclerotic and inflammatory changes produced by these hormones in the kidney and the cardiovascular system

Summary

After an introductory outline of our concepts about the relationship between inflammation and the hypophysis adrenocortical system we reviewed recent investigations in this field made by members of our Institute concerning the following topics

10 Selye H *Brit M J* 2 1129 (1949)
11 Selye H *Brit M J* Feb 10 263 (1951)
12 Guillemin R and H Selye *Ann endocrinol (Paris)* 13 835 (1952)
13 Selye H *Lancet* March 3 483 (1951)
14 Hall C E Dontigny P Beland E and H Selye *Endocrinology* 38 296 (1946)
15 Selye H *Proc Soc Exp Biol Med* 76 510 (1951)
16 Selye H *The Mechanism of Inflammation* Eds Jasmin G and A Robert Montreal Acta Inc 1953
17 Selye H *Endocrinology* 21 169 (1937)
18 Hench P S Kendall E C Slocumb C H and H F Polley *Proc Staff Meetings Mayo Clinic* 24 181 (1949)
19 Hoene R Rindani T H and G Heuser *Am J Physiol* 177 19 (1954)
20 Selye H *Canad M A J* 64 489 (1951)
21 Lemonde P Panisset M Dobija M and H Selye *J Clin Endocrinol and Metabolism* 12 973 (1952)
21a Selye H *The Story of the Adaptation Syndrome* Montreal Acta Inc 1952
22 Selye H Salgado E and J Procopio *Acta Endocrinology* 9 337 (1952)
23 Selye H *Endocrinology* 49 197 (1951)
24 Li C H and H M Evans *Recent Progress in Hormone Research* New York Academic Press Inc 3 3 (1948)
25 Mark W Simpson M E Li C H and H M Evans *Endocrinology* 33 102 (1943)
26 Selye H *Am J Physiol* 171 381 (1952)
27 Robert A and H Selye *Ann endocrinol (Paris)* 13 845 (1952)
28 Jasmin G and H Selye *Ann endocrinol (Paris)* 13 849 (1952)
29 Selye H *Oral Surg* 6 557 (1953)
30 Selye H *Ztschr Krebsforsch* (in press 1954)
31 Kass E H Lundgren M M and M Finland *J Exp Med* 99 89 (1954)
32 Salgado E and H Selye *J Clin Endocrinol and Metabolism* 12 974 (1952)
33 Selye H *J Clin Endocrinol* 6 117 (1946)
34 Selye H Presented before Third Pan American Congress of Endocrinology Santiago Chile November 21st–27th 1954
35 Selye H *Ann endocrinol (Paris)* 14 372 (1953)
36 Salgado E *Ann Rheum Dis* (in press 1954)
37 Mitchell M Guillemin R and H Selye *Endocrinology* 54 111 (1954)
38 Selye H *Ann endocrinol (Paris)* 13 841 (1952)
39 Selye H *Rev Can Biol* 9 476 (1951)
40 Stebbins R B and H C Stoerk *Am Ass Pathol and Bacteriol* 51st Ann Meeting Philadelphia Pa April 8–10 615 (1954) *Am J Path* 30 615 (1954)
41 Selye H *J Inv Dermat* 21 91 (1953)
42 Selye H *J Clin Endocrinol and Metabolism* 13 838 (1953)
43 Salgado E and H Selye (in preparation)
44 Selye H Tr Fourth Conf Nov 12 14 29 (1952) Josiah Macy Jr Found New York (1953)
45 Salgado E and H Selye *J Lab Clin Med* (in press 1954)

drawal of the hormone treatment This shows that resistance to further growth stimulation can develop although the hormone is still capable of maintaining an excessive body weight

(6) *Topical actions of STH preparations and the effects of systemic STH treatment upon the topical actions of cortisol* A number of observations indicate that STH can stimulate connective tissue growth at the site of its application Various other protein preparations do not exhibit a comparable effect, yet, the specificity of this topical action cannot be regarded as definitely proven

The topical inhibitory effect of gluco corticoids upon regional growth and inflammation is only slightly counteracted by systemic treatment with STH

(7) *Effect of STH upon the adrenals* In our experience even the highest doses of STH failed to increase the weight of the adrenals in hypophysectomized rats However if STH is given together with ACTH it increases the adrenocorticotropic effect of the latter

When ACTH is administered to intact rats it causes a pronounced adrenal enlargement (which may be due in part to a stimulation of endogenous ACTH secretion)

When ACTH is given conjointly with methylandrostenediol (MAD) to intact rats it enhances the ability of the latter to produce PAS positive cytoplasmic granules in the adrenocortical cells At the same time the production of sclerotic and inflammatory changes (nephrosclerosis arteritis myocarditis) through MAD is enhanced by STH

Acknowledgments The work reported in this communication was supported by Grant No 318 from the Defence Research Board Department of National Defence, Canada

The author is also very grateful to The Armour Laboratories (Chicago) for generous supplies of highly potent STH preparations

References

1 Selye H *The Physiology and Pathology of Exposure to Stress* Montreal Acta Inc 1950
2 Selye H *First Annual Report on Stress* Montreal Acta Inc 1951
3 Selye H and A Horava *Second Annual Report on Stress* Montreal Acta Inc 1952
4 Selye H and A Horava *Third Annual Report on Stress* Montreal Acta Inc 1953
5 Selye H and G Heuser *Fourth Annual Report on Stress* Montreal Acta Inc 1954
6 Selye H *Can M A J* 50 426 (1944)
7 Hall C E and H Selye *Rev can biol* 4 197 (1945)
8 Selye H and E I Pentz *Can M A J* 49 264 (1943)
9 Selye H Sylvester O Hall C E and C P Leblond *J A M A* 124 201 (1944)



FIG 1 a Normal pancreatic rudiment of foetal rat b Similar rudiment after 10 days cultivation *in vitro* (Chen s method) Note enlargement and advanced differentiation of the gland c High power view of the same explant showing secretory granules duct and an islet of Langerhans (After Chen 1955)

used concentrations of L thyroxine which fell within the limits encountered in the blood of human beings suffering from hyperthyroidism

Our first experiments were made on the cartilaginous long bone rudiments of 6-day chick embryos which in normal medium grow and develop very well The addition of L thyroxine to the medium in a concentration of 16 μg/100 ml had a well marked effect on such explants but different rudiments were affected in different degrees (Fig 2) Three types of effect were noted accelerated maturation of the cartilage retardation of growth and in the later stages of cultivation cellular degeneration Accelerated maturation was greatest in the wing bones and especially in the radius while retardation of growth was most pronounced in the femur and tibia (Figs 3 4)

In other experiments the mesodermal condensation or blastema destined to form the limb skeleton was removed from younger (4½ day) buds and cultivated in the same way The main effect of the thyroxine in a concentra

8

The Effect of Hormones on Differentiated Tissues in Culture

Honor B Fell
Strangeways Research Laboratory Cambridge England

It is now possible to cultivate *in vitro* many different types of organ rudiments in such a way that they preserve their characteristic morphology and often exercise their normal functions Bones teeth hair endocrine and exocrine glands the respiratory tract and the gonads have all been grown in an organised state Even the mammalian foetal pancreas (Figs 1 a b c) has recently been grown as an organ culture by my colleague Dr Chen using a method of his own devising [1]

The advantages of such cultures for the study of hormonal activity are obvious but so far they have not been very extensively used for this purpose Most of the research on these lines is being done in three departments viz by Professor Gaillard and his colleagues in the Department of Experimental Histology at Leiden by Professor Etienne Wolff and his group at the Department of Embryology in Strassbourg and by ourselves at the Strangeways Laboratory in collaboration with Sir Edward Mellanby of the National Research Institute London As time is limited I have been asked to confine my talk to our own work in Cambridge and London

The experiments I am going to describe relate to four types of hormones (1) the thyroid active principles (2) the growth hormone (3) insulin and the hyperglycaemic factor and (4) sex hormones I will deal with the results in this order

Thyroid Active Principles Recently there has been some discussion as to whether thyroxine itself has a direct action on the target organs or whether it has first to be transmuted into tri iodothyronine In collaboration with Sir Edward Mellanby experiments were made to see whether thyroxine would have any effect on organ cultures *in vitro* [2] We selected as our material the skeletal rudiments from the limb buds of chick embryos and we

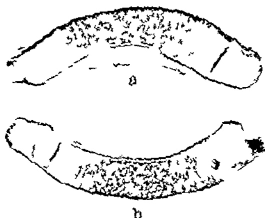


FIG 4 a Control ulna from the same embryo as the tibiae shown in Fig 3 6 days in normal medium b Opposite ulna from the same chick after 6 days in medium containing 16 µg 100 ml of added L thyroxine The ulna is less susceptible to the injurious action of the hormone than the tibia the hypertrophic cartilage is rather more advanced than in the control but the explant is otherwise unaffected (After Fell and Mellanby 1955)

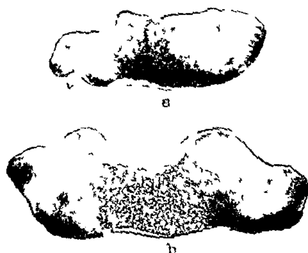


FIG 5 a A control skeletal blastema from the wing bud of an embryo of Group I after 9 days cultivation in normal medium The humerus shows no chondroblastic hypertrophy or ossification b The opposite wing blastema from the same chick after 9 days growth in medium containing 16 µg/100 ml of added L thyroxine The development of the explant has been stimulated it is larger than its control in the shaft of the humerus the cartilage cells have undergone hypertrophy and periosteal ossification has begun

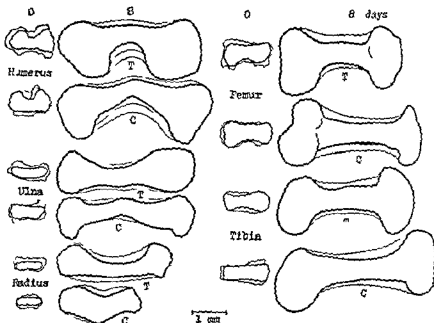


FIG 2 Camera lucida drawings of a set of living long bone rudiments from the same 6 day embryonic chick One of each pair was grown for 8 days in medium containing 16 μg thyroxine per 100 ml and the other in control medium Note the differential effect of the hormone on the growth of the various rudiments as compared with the controls the shaft is much shorter in the treated humerus femur and tibia slightly shorter in the treated ulna but longer in the treated radius (After Fell and Mellanby 1955)

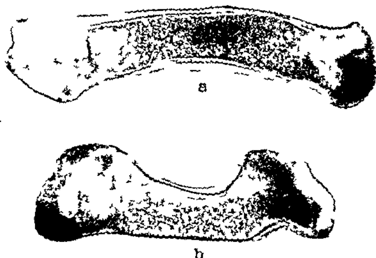


FIG 3 a A control tibia from a 6 day embryonic chick after 6 days in normal medium the cartilage cells of the shaft have hypertrophied and periosteal bone has been formed b The opposite tibia from the same embryo after 6 days in medium containing 16 μg/100 ml of added L thyroxine The shaft is much shorter than in the control but shows advanced chondroblastic hypertrophy and periosteal ossification (After Fell and Mellanby 1955)

by indirect means or alternatively avian rudiments may not be capable of responding to its action A suitable method for cultivating foetal rat bones has now been developed by Miss Hay and the experiments will be repeated on this material

Insulin and the Hyperglycaemic Factor Although explanted avian skeletal rudiments are unaffected by the growth hormone they respond readily to insulin This was shown by Dr Chen[4] who found that when the long bone rudiments of 6–7 day chick embryos were cultivated in medium containing 0 16 unit of insulin/ml the growth of the shaft was retarded while the epiphyses were greatly enlarged (Fig 6) The explant became soft and flexible and in contrast to thyroxine insulin impeded the maturation of the cartilage so that the zones of proliferative and hypertrophic cells were poorly differentiated Dr Chen found that embryo extract inactivates insulin and consequently the action of the hormone was much more pronounced when the extract was omitted from the medium (Fig 7) Under these conditions a concentration of only 0 0016 unit/ml produced a significant effect on the rudiments

It will be seen from the curves in Fig 7 that like thyroxine insulin affected different rudiments in different degrees the humerus and tibia were the most susceptible to its influences and the radius the most resistant

The effect of the hyperglycaemic factor on the explanted rudiments was also investigated but negative results were obtained

At the present time the action of insulin is being studied on a completely different type of tissue viz on the brown fat of the foetal rat A young American from Harvard Dr Sidman is making these experiments in our Laboratory In the animal an increased quantity of glycogen appears in the brown fat cells under various physiological conditions including treatment with insulin When the rudiments of the brown fat are cultivated in normal fowl serum glycogen is transiently deposited in the tissue and lipid is gradually formed After the addition to the culture medium of 4 0 γ/ml of insulin virtually free from the hyperglycaemic factor the amount of glycogen deposited is greatly increased and may equal or even exceed that induced by the hormone in the same tissue *in vivo* A similar effect though less pronounced has been obtained with cultures grown in Dr Raymond Parker s artificial medium No 770

Sex Hormones We come now to the direct action of sex hormones on organ cultures

In Australia a former member of our group Dr Margaret Hardy in collaboration with J D Biggers and P J Claringbold showed that the vaginal epithelium of the young mouse when cultivated *in vitro* and treated with oestrogen keratinises[5] An American in our Laboratory Dr Raymond Kahn (now in Professor Patten s Department at Ann Arbor) had independently undertaken a similar research and using vaginal explants from young rats confirmed the Australian workers results[6] Dr Kahn found,

tion of 16 μg/100 ml and even of 1 μg/100 ml was to promote and accelerate differentiation Very little degeneration was produced Here again different rudiments reacted in different degrees, stimulation being greatest in the humerus (Fig 5)

From these results we concluded that thyroxine does act directly on the target organ The changes produced by the hormone *in vitro* viz accelerated maturation of the cartilage and inhibition of growth seem comparable with the precocious development premature ageing and reduced growth of the bones in young animals treated with thyroxine [3]

Experiments are now being made with tri iodothyronine, which is found to be similar to thyroxine in its effect but nearly four times as potent

Growth Hormone Since this conference is primarily concerned with the growth hormone I should have liked to describe some dramatic results with this agent but unfortunately that is not to be! In collaboration with the Department of Biochemistry Cambridge University my colleagues Dr Chen and Miss Hay have made a long series of experiments in which various fractions and amounts of growth hormone have been added to the culture medium in which the long bone rudiments of 6 day chick embryos were grown With one doubtful exception only negative results were obtained which suggests that the growth hormone does not have a direct effect on chick skeletal rudiments The reason for this is not yet known it may be that *in vivo* the growth hormone produces its effect on the skeleton

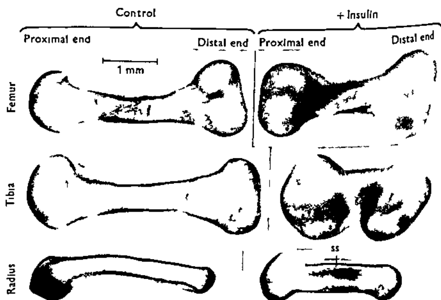


Fig 6 Photographs of living explants of bones from a 7 day chick embryo after 8 days *in vitro* one of each pair was grown in control medium (plasma clot without embryo extract) and the other in medium to which insulin (0 16 unit/ml) had been added Note the enlarged ends and very short shafts of the treated rudiments (After Chen 1954)

In control medium the glands from the young animals showed some loss of epithelial differentiation (Fig 8) but those from 6 months old animals preserved their normal differentiation (Fig 9) Dr Lasnitzki concluded that to maintain their normal structure, the young glands require a larger supply of androgenic hormone than is available in the normal culture medium while older glands are either independent of such hormones or require less

She tested this view by adding testosterone propionate to the culture medium Under these conditions the young prostate preserved its characteristic structure but more prolonged exposure caused the epithelium to become hyperplastic The same dose of testosterone propionate added to the



Fig 8 Prostate gland from a young (4–6 weeks) mouse grown in control medium for 10 days The explant shows some loss of epithelial differentiation the alveoli are rather dilated and the epithelium is somewhat flattened New alveoli are formed at the periphery (After Lasnitzki)



Fig 9 Prostate gland from an older (6 months) mouse after 10 days cultivation in control medium The normal structure of the epithelium has been preserved *in vitro* (After Lasnitzki)

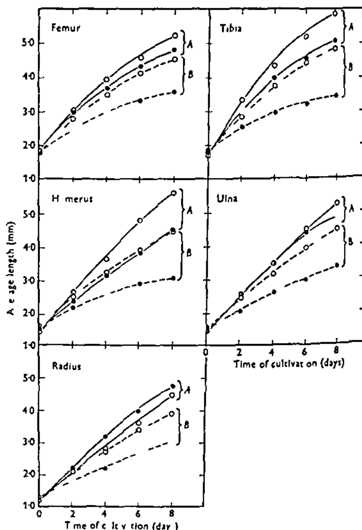


FIG 7 The effect of insulin on the average length of the bone rudiments cultivated *in vitro* A explants cultivated in medium containing embryo extract B explants cultivated in medium without embryo extract O–O control ●–● insulin treated (After Chen 1954)

however, that his controls in normal medium also keratinised after more prolonged cultivation This however could be prevented by adding 3000 I U /100 ml of synthetic vitamin A to the medium Under the conditions of the experiment this concentration of vitamin A retarded but did not inhibit keratinisation in the oestrogen treated cultures

My colleague, Dr Lasnitzki has investigated the effect of sex hormones on explants of the prostate glands from mice of two age groups viz 4–6 weeks and 6 months She found that the behaviour of the glands in normal medium and their response to male and female hormones largely depended on the age of the animal from which they were taken

medium of older glands produced a much more rapid hyperplasia with mitotic stimulation (Fig 10) many of the mitotic figures in these hyper plastic glands were abnormal

In other experiments Dr Lasnitzki investigated the effect of oestrone on the explanted prostates Here again she found a difference in response be tween the younger and older glands A concentration of 2γ/ml of oestrone in the culture medium of young prostates caused hyperplasia and squamous metaplasia of the epithelium but had little effect on the stroma (Fig 11) When older glands were grown in medium containing 2γ/ml of oestrone epithelial hyperplasia was rarely produced but the amount of fibromuscular stroma was greatly increased (Fig 12) Dr Lasnitzki has pointed out that this response recalls the benign enlargement of the prostate in elderly men which is due to a hypertrophy of the connective tissue and thus supports the view expressed by Burrows[3] that this condition may have an oestrogenic origin

In this brief account I have summarised the recent results which my colleagues and I have obtained in our work on the direct action of hormones on organ cultures *in vitro* So far we have been mainly concerned with the morphological response of the tissues under various experimental condi tions but we hope shortly to investigate the biochemical basis of some of these histological reactions



with 2γ/ml of oestrone
but the density of the



FIG 10 Similar explant from a 6 months old mouse after 10 days cultivation in medium containing 50γ/ml of testosterone propionate The epithelium has undergone rapid hyperplasia (After Lasnitzki)



FIG 11 Young prostate gland grown for 3 weeks in medium to which 2γ/ml of oestrone had been added Note the squamous metaplasia of the epithelium and occluded alveoli the stroma is unaffected (After Lasnitzki 1954)

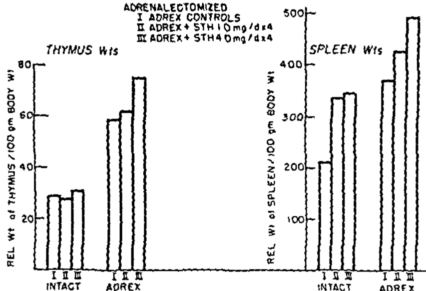


Fig 1

found that the animals receiving growth hormone acquired rather large spleens This effect is considerably different than the effect of the hormone on other organized lymphatic tissue structures In Figure 1 are shown certain data of such a study with two different doses of the hormone I will concentrate on just one dose comparing the response in the intact and the adrenalectomized controls with that in the adrenalectomized growth hormone treated animals You will note that with the large dose of STH there is a slight increase in thymus size Note, however that the size of the spleen is much greater in the adrenalectomized STH treated animals There is also a marked increase in the size of the spleen in the intact growth hormone treated animals whereas there was no change in size of thymus or lymph nodes This observation was of real interest when we considered that frequently in acute infections there is a moderate increase and sometimes quite a great increase in the size of the spleen This of course indirectly suggested a possible direct action of STH on the spleen

In Figure 2 is demonstrated the dose response relationship between the logarithm of the dose of growth hormone and the weight of the spleen the hormone having been administered over a four day period This relationship has been obtained for hypophysectomized rats for intact rats and for

Acknowledgment I am indebted to the editors of the following Journals for permission to reproduce figures *Experimental Cell Research* (Fig 1) *Journal of Physiology* (Figs 2–7) and *Cancer Research* (Fig 11)

References

1 Chen J M *Exp Cell Research* (in press 1955)
2 Fell H B and E Mellanby *J Physiol* (in press 1955)
3 Simpson M E Asling C W and H M Evans *Yale J Biol and Med* **23** 1 (1950)
4 Chen J M *J Physiol* **125** 148 (1954)
5 Hardy M H Biggers J D and P J Claringbold *Nature* **172** 1196 (1953)
6 Kahn R H *Nature* **174** 317 (1954)
7 Lasnitzki I *Cancer Research* (in press 1955)
8 Burrows H *Biological Action of Sex Hormones* New York and London Camb Univ Press 1949

DISCUSSION

Effects of Growth Hormone on Certain Structures

Designated Discussion

THOMAS F DOUGHERTY (University of Utah School of Medicine) With respect to the discussion of the papers presented this afternoon I would like to confine my remarks particularly to the so-called prophlogistic effect of growth hormone or somatotropin (STH) First of all some years ago in our laboratory we demonstrated that desoxycorticosterone antagonizes the anti inflammatory or antiphlogistic effect of cortisone In this concept we are in complete agreement with Dr Selye It was further evident that this antagonism is not due to pituitary suppression or to inhibition of adrenal output since this effect is evident in both hypophysectomized and adrenalectomized animals The inhibitory effect or the antagonistic effect of desoxy corticosterone on certain actions of cortisone was demonstrated also in its property of diminishing the anti anaphylactic effect of cortisone Thus one can markedly moderate the antiphlogistic and anti anaphylactic actions of cortisone by the simultaneous administration of desoxycorticosterone We demonstrated further in the acute inflammatory response that desoxycorticosterone and cortisone interacted on a mol to mol basis and we postulated a type of competitive action and inhibition in the tissues No such demonstration of action has been obtained as yet for STH Consequently, we feel that the action of STH in reducing the anti inflammatory effect of cortisone or hydrocortisone is probably mediated by a different mechanism than that which is operative in the action of DCA In studying this phenomenon we ran across a side issue which I would like to discuss for a few minutes

In the course of our studies with growth hormone on the potentiation of antibody production and on its relationship to anaphylactic shock we

significant increase in the plasma volume of the STH treated animal Consequently, it is apparent that an actual normocytic anemia exists in relation to the increase in plasma volume This led us to the supposition that possibly the increase in plasma volume resulting from the growth hormone is taken up in large part by the spleen with an accompanying sequestration of red blood corpuscles This can be demonstrated best in splenectomized animals

Now the increased erythropoiesis in bone marrow is accompanied by a marked increase in the number of immature red corpuscles The normoblastic series is markedly increased and the nucleus in certain cells is quite open I am not saying that these cells are megaloblasts but the similarity is quite apparent To claim that one can produce a megaloblast in an animal is to place oneself in a most precarious position

The other observation of great interest is the considerable plasma cell development which occurs throughout the tissues of STH treated animals There is tremendous development of such cells in the spleen This observa

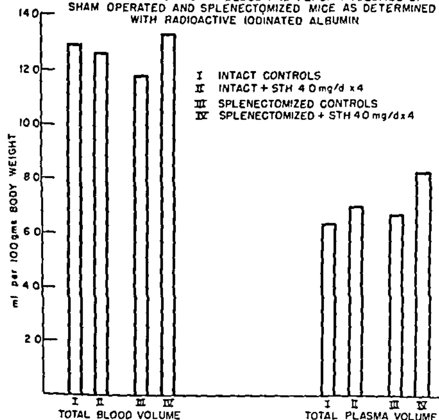


FIG 3

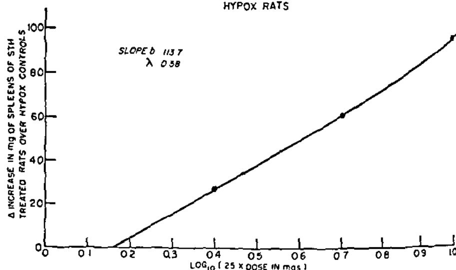


Fig 2

intact mice It is possible that it could be used as an assay We do not know enough about it as yet except that we have obtained a similar slope with two different batches of hormone in two different species of animals whether they are hypophysectomized or intact

Now these observations that administered growth hormone increases the size of the spleen led to the study of the mechanism In our histological sections there is reticulum cell hyperplasia and a good many macrophages or fixed reticulo endothelial elements There are also many highly basophilic cells which we call histiocytes The splenic sinuses are stuffed by this I mean stuffed like blood sausage with red blood corpuscles Much of the increase in splenic size is due to this blood stuffing rather than to any actual growth of spleen meat Accordingly we feel that this increase is related also to sequestration of red cells At the same time we found in the bone marrow an increase in red cell production We could not pick up any evidence in the peripheral blood of this increased erythropoiesis using the mean corpuscular hemoglobin concentration red cell count etc as our indices This finding led us to consider the possibility that perhaps we were observing an increase in plasma volume which was obscuring the picture as far as blood indicies were concerned Accordingly plasma volumes were obtained on mice, both splenectomized and sham operated, using albumin tagged with radioactive iodine As noted in Figure 3 the intact mice given STH had plasma volumes which were not significantly different from those of the intact untreated controls Splenectomized controls had a small decrease in blood volume while splenectomized STH treated animals had a small but insignificant increase in blood volume as compared to the intact controls If we take out the spleen however we find that we have a highly

with us one of the best tissue culturists in the person of Dr Fell it is very important I think to ask her whether or not there is any well documented *in vitro* experiment in which growth hormone has induced the growth of any tissue

FREDERICK HISAW (Chairman) I know we all would be glad to hear your opinion on that Dr Fell

HONOR FELL Well I don t know of anything There have been some experiments reported by Ver Dam in Holland on organ cultures of rat bones but we were not wholly satisfied with them ourselves He described an effect but not one on the growth of the bones He maintained that they differentiated better with the growth hormone yet there was no increase in length whatsoever I don t know of any other such study with growth hormone which is convincing

FRANK ENGEL I was very much interested in Dr Fell s observations in respect to the influence of insulin on adipose tissue Could she tell us just a little bit more as to how long the cultures were exposed to insulin prior to the development of the marked increase in glycogen? Was this an acute effect or was this an effect which was sustained over many days? I am interested in this aspect because many people have postulated that the deposition of glycogen in adipose tissue in response to insulin and other stimuli is a phenomenon which precedes fat deposition I am interested to know also whether Dr Fell has made any observations on the effects of cortisone in this response of adipose tissue to insulin We found rather unexpectedly that although cortisone had no effect on the concentration of glycogen in the tissue when animals pre treated with cortisone were given insulin there was a much greater increase in glycogen in the tissue than from insulin alone This was a little surprising in view of the fact that cortisone is reputed to inhibit fat synthesis and in view of the glycogen deposition occurring before this It would be of interest to know whether she feels that the insulin had any growth promoting effect on this adipose tissue

HONOR FELL Well the effect of the insulin was a transitory thing which was succeeded by fat formation Incidentally I should emphasize that the work is being done not by myself but by my young colleague Dr Sidman In regard to your other point insulin does appear to improve both growth and survival of the adipose tissue Dr Sidman finds that with more prolonged cultivation the insulin treated cultures are always very much healthier They seem to be larger and better altogether than those without insulin

tion confirms the work of Cavellero who has demonstrated a similar plasma cell increase in various organs of STH treated animals We have found this change particularly prominent in the bone marrow I should like to emphasize that we have found also a shift to the left in the marrow cells and a marked maturation arrest mostly at the level of the basophilic erythroblast

Thus I would like to sum up these few findings in the sense that we feel STH has a marked stimulatory effect on immature erythropoiesis This effect we believe is on actual growth or on cell mitosis with an increase in the number of immature cells without an accompanying differentiation Concomitantly there is an increase in plasma volume which is obscured by sequestration in the spleen of probably both plasma and cells

General Discussion

T LEVITT (New End Hospital England) One could not help but be impressed by the galaxy of interesting and important papers I would like to ask one question which is directed to Dr Baker Did he find any correlation between the form and function of the exocrine glands and those of the endocrine glands in the patients he has studied? Our field has widened greatly and it is important to assess the relationship of not only endocrine to endocrine but endocrine to exocrine glands

BURTON L BAKER Firstly let me emphasize that all of our work was performed in rats and not in patients Secondly as far as the assessment of the relationship of these glands to the endocrine glands is concerned in any experiment one can vary only so many factors The endocrine factor we varied in the beginning of the experiments either by taking out a gland or a combination of glands or by introducing a hormone into a hypophysectomized animal Coming to your specific question we have not studied the effects of these hormonal treatments on the other ductless glands but I can assure you that the literature is filled with such observations

ERIC REID With reference to the absence of an effect of growth hormone in the studies from Dr Fell s laboratory I would like just to mention an observation made in Glasgow by Dr Leslie He has cultured chick heart fibroblasts and exposed them to certain hormones Growth hormone alone did little Cortisone alone did little When these hormones were given together however there was marked cell proliferation That may be a clue

HONOR FELL Well one of the things we have on our agenda is to evaluate the effects of two hormones together, and we have planned particularly to study thyroxin and growth hormone It would be very interesting to try cortisone too I ll bear that in mind

BURTON L BAKER Since the chemists have had some doubt today as to whether or not there is such a thing as growth hormone and since we have

with us one of the best tissue culturists in the person of Dr Fell it is very important I think to ask her whether or not there is any well documented *in vitro* experiment in which growth hormone has induced the growth of any tissue

FREDERICK HISAW (Chairman) I know we all would be glad to hear your opinion on that Dr Fell

HONOR FELL Well I don t know of anything There have been some experiments reported by Ver Dam in Holland on organ cultures of rat bones but we were not wholly satisfied with them ourselves He described an effect but not one on the growth of the bones He maintained that they differentiated better with the growth hormone yet there was no increase in length whatsoever I don t know of any other such study with growth hormone which is convincing

FRANK ENGEL I was very much interested in Dr Fell s observations in respect to the influence of insulin on adipose tissue Could she tell us just a little bit more as to how long the cultures were exposed to insulin prior to the development of the marked increase in glycogen? Was this an acute effect or was this an effect which was sustained over many days? I am interested in this aspect because many people have postulated that the deposition of glycogen in adipose tissue in response to insulin and other stimuli is a phenomenon which precedes fat deposition I am interested to know also whether Dr Fell has made any observations on the effects of cortisone in this response of adipose tissue to insulin We found rather unexpectedly that although cortisone had no effect on the concentration of glycogen in the tissue when animals pre treated with cortisone were given insulin there was a much greater increase in glycogen in the tissue than from insulin alone This was a little surprising in view of the fact that cortisone is reputed to inhibit fat synthesis and in view of the glycogen deposition occurring before this It would be of interest to know whether she feels that the insulin had any growth promoting effect on this adipose tissue

HONOR FELL Well the effect of the insulin was a transitory thing which was succeeded by fat formation Incidentally I should emphasize that the work is being done not by myself but by my young colleague Dr Sidman In regard to your other point insulin does appear to improve both growth and survival of the adipose tissue Dr Sidman finds that with more prolonged cultivation the insulin treated cultures are always very much healthier They seem to be larger and better altogether than those without insulin

9

Growth Hormone Induced Bone and Joint Changes in the Adult Rat

*C W Asling M E Simpson, H D Moon, C H Li and H M Evans**

Institute of Experimental Biology University of California Berkeley†

The demonstration by Evans and Long in 1921–1922[1 2] that chronic administration of pituitary extracts produced gigantism in experimental animals confirmed the conception that this disorder in human beings results from pituitary hyperfunction It is well known that generalized overgrowth or gigantism in the human may eventually be associated with disproportion of skeletal parts and localized bony overgrowths which are among the most characteristic features of acromegaly

Changes resembling acromegaly have been produced experimentally by pituitary extracts in several animal forms in the dog by Putnam Benedict and Teel[3] and by Evans Simpson Meyer and Reichert,[4] in the guinea pig and mouse by Silberberg and Silberberg[5] Selye[6] had reported that growth hormone sensitizes rats to the development of topical irritation arthritis Reinhardt and Li[7] have reported arthritic changes in the knee and ankle joints of gonadectomized adrenalectomized rats treated for a prolonged period with purified growth hormone in high dosage

The detailed studies of the effect of long continued administration of growth hormone on skeletal growth in the rat have recently been summarized by Simpson Asling and Evans[8] It was concluded that the gigantism resulting in rats from growth hormone was primarily the result of a proliferative action without accompanying advance in differentiation of the

---

* Read by Dr Miriam E Simpson

† Aided by grants from the U S Public Health Service RG-409 A 366 and C-1098 the Research Board of the University of California the American Cancer Society Inc New York and the University of California Cancer Grant

skeleton by closure of the epiphyses Certain of the epiphyseal centers in the rat remain open and responsive to growth hormone late in life and any disproportions in growth which may appear in the prolonged action of excess amounts of growth hormone could conceivably be due to the continued activity at these centers inasmuch as the other centers disappear and hence no longer participate in growth Certain abnormalities in growth of the skeleton other than those dependent on epiphyseal response have been observed however in experimental gigantism[9] Among these is excess periosteal osteogenesis as manifested by excessive thickness of the cortex of long bones and the prominence of their tuberosities (e g the deltoid) Similarly the skull laminae and diploe are thickened and the muscular attachments are increased in ruggedness[10]

Other changes were observed involving both the dense connective tissues and bone which could not be classified simply as exaggerated normal phenomena These fell instead into the category of distortion or disease It was noted for example that chronically injected rats assumed a humped position and that the spine could not be straightened completely after anesthesia or even after death Occasional transient episodes of redness and swelling of an ankle were noted The thorax was distinctly misshapen in many injected rats it was wider and deeper and the lower rib margin flared the xiphoid was markedly everted and the costochondral junction was thickened and displaced laterally There was also a generalized stimulus to the growth of fibrous connective tissues resulting in thickened dermis of the skin and increased size of fibers in tendons and ligaments (Evans Simpson and Li)[11 1]

It is proposed here to examine the skeletal abnormalities induced in the rat by growth hormone with particular emphasis on the changes in and about the joints in order to determine whether or not they represent acromegalic osteoarthropathy The analysis deals chiefly with the bones forming the knee and ankle joints and with the vertebral column where the majority of joint changes occurred The significant abnormalities were demonstrable in roentgenograms Histologic examination has contributed to the understanding of the pathogenesis and to the recognition of the growth hormone induced osteoarthropathy as an entity distinct from other types of joint derangement

The present report describes the bone and joint changes of rats of the same groups on which a previous report was made regarding the high incidence of neoplasms after prolonged treatment with growth hormone (Moon et al)[13 14] The rats were females 237–239 days of age which had reached the growth plateau Thus in their skeletons only those epiphyseal centers were patent which persist into old age The groups initially included 15 each of hypophysectomized or intact experimental animals and their respective controls An interval of 2 weeks was allowed to elapse between hypophysectomy and onset of the experiment in order to eliminate incompletely hy

pophysectomized animals Highly purified pituitary growth hormone was injected for over a year in each group, the hypophysectomized rats were treated for a shorter period than the intact rats (380 as compared with 480 days) and accordingly received a lower total amount of hormone The daily dose was increased at intervals during the experiment, as may be read from Figures 1 and 2 Hypophysectomized and normal controls were injected with an inert protein human serum albumin Although it was necessary to sacrifice a few animals before the end of the injection period half or more of each group was treated and observed for the full period No animal is included in this study which had not been treated for at least 10 months No operated animals have been considered which did not meet the usual criteria for completeness of hypophysectomy, i e a sella turcica free of epithelial tissue and histological evidence of atrophic endocrine organs

Figures 1 and 2 serve to demonstrate in terms of body weight, the gigantism which resulted in the injected groups The first graph shows the initial weight the doses used the duration of injection, and the body weight increase of the hypophysectomized rats The lack of growth of controls is

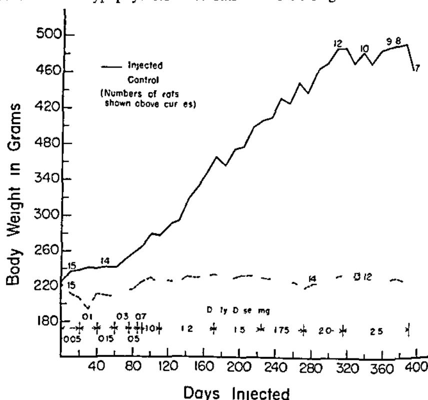


FIG 1 Curves showing body weights of adult hypophysectomized female rats injected chronically with pituitary growth hormone at doses indicated compared with protein injected controls

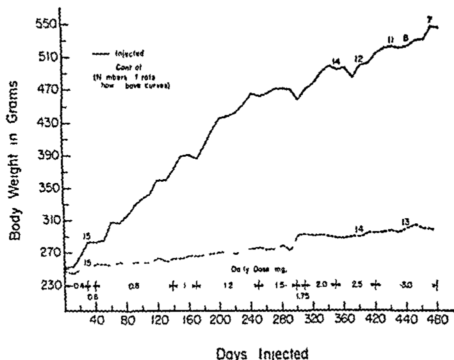


Fig 2 Curves showing body weights of intact adult female rats injected chronically with pituitary growth hormone at the doses indicated compared with protein injected controls

indicated by the lower curve The second graph gives similar data for the intact animals treated and control

In Table 1 is shown the overgrowth in total body length (tip of nose

Table 1

MEAN LENGTHS OF BODY AND OF TIBIA IN FEMALE RATS AFTER CHRONIC INJECTION WITH GROWTH HORMONE

Groups	No of Rats	Body Length		Tibia	
		Total (cm)	Increase (%)	Length (mm)	Increase (%)
HYPOPHYSECTOMIZED					
Controls	13	37 4 ± 0 4*		38 5 ± 0 4	
Growth Hormone	14	44 3 ± 0 8		45 8 ± 0 8	
			11 8		11 9
NORMAL					
Controls	15	40 7 ± 0 7		40 0 ± 0 2	
Growth Hormone	15	44 7 ± 0 4		44 0 ± 0 3	
			11 0		11 0

* Standard error



FIG 3 Skulls of normal adult female rat (A) and intact growth hormone injected rat (B) Increased dimensions ruggedness especially at the attachment of suboccipital and temporal muscles (× 2 25)

to end of tail) and in the length of a representative bone the tibia which occurred in growth hormone injected hypophysectomized and intact rats The body length is largely attributable to vertebral growth In treated rats of both types it exceeded that of the respective controls by about 11 per cent The increase in length of the appendicular skeleton as indicated by the tibial length was of the same order

It is of interest that the incidence of neoplasms previously reported was found to be higher in the intact growth hormone treated rats, whereas it will be shown here that the bone and joint abnormalities were more pronounced and numerous in the hypophysectomized treated rats

A survey of the skeleton by roentgenograms showed that joint changes were most frequent and deforming at the knee ankle, and in the vertebral column There were also certain characteristic changes in the skull and mandible which are recorded in view of the pronounced changes which occur in the skull of the human acromegalic with which the experimentally induced changes in the rat will be compared

The increased length and breadth of skull of the injected rat with increased height of the ridges constituting the regions of muscular attachment can be seen in Figure 3 A section through the exaggerated temporal crest of such an animal shows the marked increase in height of the muscular attachment as contrasted with the control (Fig 4) The increase in thickness of the laminae is also clearly shown in these sections The hematopoietic marrow has been expanded within the diploe and even extended into the laminae

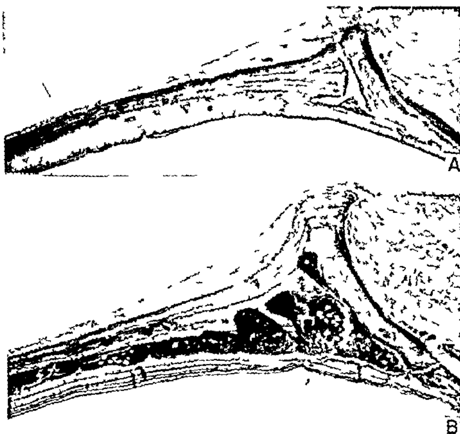


FIG 4 Transverse section through the parietal bone of growth hormone treated intact rat (B) Increased thickness of bone and prominence of temporal crest as compared with the control (A) (H & E × 18 75)



FIG 3 Skulls of normal adult female rat (A) and intact growth hormone injected rat (B) Increased dimensions ruggedness especially at the attachment of suboccipital and temporal muscles (× 2 25)

to end of tail) and in the length of a representative bone the tibia which occurred in growth hormone injected hypophysectomized and intact rats The body length is largely attributable to vertebral growth In treated rats of both types it exceeded that of the respective controls by about 11 per cent The increase in length of the appendicular skeleton as indicated by the tibial length was of the same order

It is of interest that the incidence of neoplasms previously reported was found to be higher in the intact growth hormone treated rats whereas it will be shown here that the bone and joint abnormalities were more pronounced and numerous in the hypophysectomized treated rats

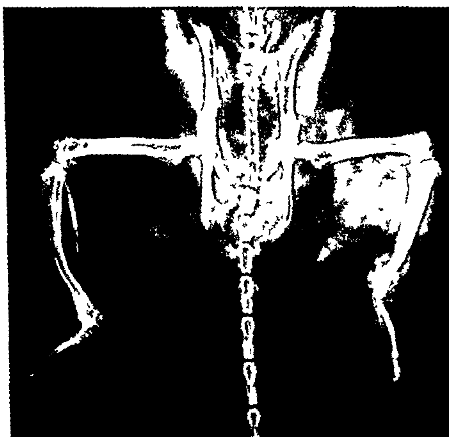


Fig 6 Roentgenogram of the lower extremities of an hypophysectomized adult female rat which had received prolonged treatment with growth hormone Note bilateral abnormalities at knee and ankle (Natural size)

rat the lower molar misses the corresponding upper molar entirely The relation between the incisors has also been changed In the rat these teeth continue to grow throughout life and are continuously worn down by action one on the other Normally the inner margin of the upper incisor is eroded at an obtuse angle in the incisor of the treated rat this angle is no longer present due to the antero-displacement of the mandible

In the more severely affected animals both knee and ankle joints were disfigured as seen in Figure 6 The bilateral symmetry of changes seen here was characteristic of all of the knee and ankle deformities The irregularities in bony surfaces present in the particular animals the joints of which are depicted are so numerous and marked as to render difficult the complete analysis of the changes These joints were by no means always so extensively altered and it was possible to classify the degree of damage The injury to the knee was graded from roentgenograms as slight, moderate or marked by the following criteria

The greater size of the mandible in the growth hormone injected rat and the resulting dental malocclusion are seen in a lateral view of the skulls (Fig 5) The increase in length of the mandible has previously been shown to result from stimulation of chondrogenesis and osteogenesis at the condylar cartilage [10 1] Although it is not possible to speak of prognathism in the rat characteristic dental malocclusion resulted from this disproportionate growth of the mandible It is also to be noted that in the normal rat the first lower molar articulates with about half of the first upper molar as a result of mandibular antero displacement in the growth hormone injected



FIG 5 Lateral view of rostral part of skulls of normal adult female rat (A) and growth hormone injected rat (B) Elongation of mandible molar malocclusion and change in shape of inner margin of upper incisor in the injected rat (X 2 25)

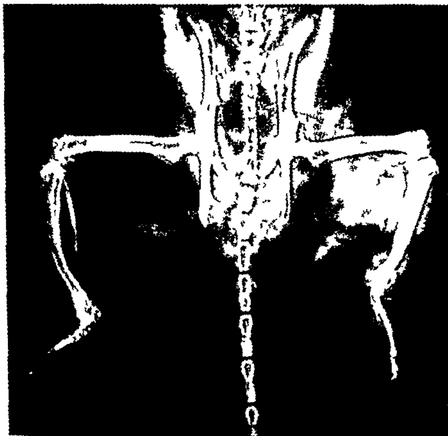


FIG 6 Roentgenogram of the lower extremities of an hypophysectomized adult female rat which had received prolonged treatment with growth hormone Note bilateral abnormalities at knee and ankle (Natural size)

rat the lower molar misses the corresponding upper molar entirely The relation between the incisors has also been changed In the rat these teeth continue to grow throughout life and are continuously worn down by action one on the other Normally the inner margin of the upper incisor is eroded at an obtuse angle in the incisor of the treated rat this angle is no longer present due to the antero-displacement of the mandible

In the more severely affected animals both knee and ankle joints were disfigured as seen in Figure 6 The bilateral symmetry of changes seen here was characteristic of all of the knee and ankle deformities The irregularities in bony surfaces present in the particular animals the joints of which are depicted are so numerous and marked as to render difficult the complete analysis of the changes These joints were by no means always so extensively altered and it was possible to classify the degree of damage The injury to the knee was graded from roentgenograms as slight moderate or marked by the following criteria

The greater size of the mandible in the growth hormone injected rat and the resulting dental malocclusion are seen in a lateral view of the skulls (Fig 5) The increase in length of the mandible has previously been shown to result from stimulation of chondrogenesis and osteogenesis at the condylar cartilage [10 1] Although it is not possible to speak of prognathism in the rat, characteristic dental malocclusion resulted from this disproportionate growth of the mandible It is also to be noted that in the normal rat the first lower molar articulates with about half of the first upper molar as a result of mandibular antero displacement in the growth hormone injected



Fig 5 Lateral view of rostral part of skulls of normal adult female rat (A) and growth hormone injected rat (B) Elongation of mandible molar malocclusion and change in shape of inner margin of upper incisor in the injected rat (× 2 25)

Table 2

DEFORMITIES OF THE KNEE JOINT

Groups	Number of Rats	Degree of Deformity			
		None	Slight	Moderate	Marked
NORMAL					
Controls	15	15	0	0	0
Growth Hormone	14	2	8	4	0
HYPOPHYSECTOMIZED					
Controls	10	10	0	0	0
Growth Hormone	13	1	3	5	4

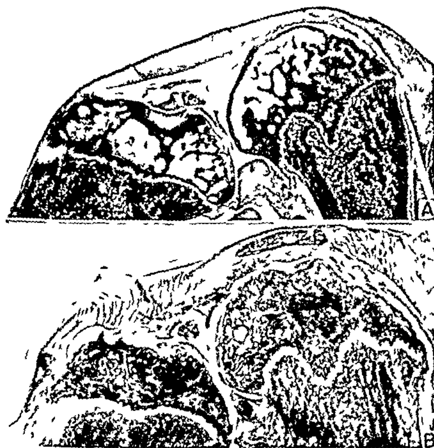


FIG 8 Histological sections of the knee joint of hypophysectomized female rats in the parasaggital plane through the medial condyles (H & E × 9) A Control injected with inert protein B Growth hormone injected Thickening and irregular outline of articular cartilage and bone underlying it increased fibrous tissue in subpatellar fat pad synovial lining and at junctional areas between periosteum cartilage and bone

1 Irregular contour of condyles of femur and tibia
2 Patellar enlargement with indefinite and irregular outline of the subcutaneous surface, and of proximal and distal borders
3 Irregular borders and enlargement of menisci and fabella
4 Malformation of proximal tibial epiphysis and tibial tuberosity
5 Appearance of an overhanging tibial epiphysis with radiolucence distal to the ventral end of the epiphyseal cartilage plate lipping
6 Increased joint space (due to hypertrophy of cartilage)

In Figure 7 are shown a series of extremities representing the progressive degrees of deformity of the knee joint

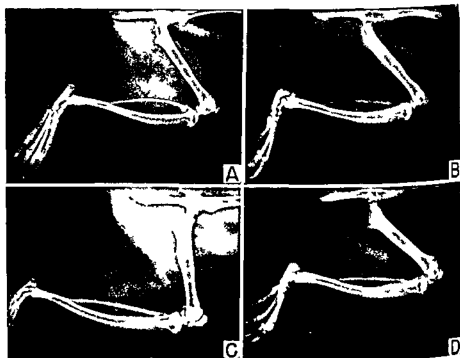


FIG 7 Roentgenograms of the knee joint of hypophysectomized adult female rats (× 0 75) A Control injected with inert protein B C D Growth hormone injected Slight moderate and severe degrees of deformity

The incidence of deformity of the knee joints as judged by roentgenograms is summarized according to severity in Table 2 No significant deformities were seen in any of the untreated rats normal or hypophysectomized The deformities were more common and usually more severe in the hypophysectomized rats injected with growth hormone than in injected animals possessing a pituitary

Histologic sections presented in Figure 8 show the knee joints of a hypophysectomized control and of a growth hormone treated hypophysec

tral aspect of the tibial diaphysis in the region where radiolucence gave the picture of an overhanging epiphysis there is a great increase in fibrous tissue in the junctional area between periosteum cartilage, and bone In the con trol the bony end of the diaphysis is closer to the epiphyseal plate and more sharply defined

The larger size of the patella in the injected rat can be seen in Figure 8 but in the mid sagittal section of the same joint in Figure 9 the change in the patella is shown to better advantage Irregular outgrowths from the surface of the patella are seen to extend into the surrounding connective tissue whereas in the control the contour is discrete In this figure is shown to ad vantage the increased connective tissue at the junctional area which in this case developed on the ventral surface of the femur The extension of bone into this fibrous area is also well demonstrated

At the ankle joint there are two types of changes one developing in the bones of the joint and the other in adjacent ligaments These changes do not always parallel one another in intensity In the series of roentgenograms in Figure 10 are shown the progressive degrees of irregularity of bony outline at the ankle especially that involving the tibia and calcaneus In the extreme the radiopacities are so diffuse and irregular that the individual bones can not be defined

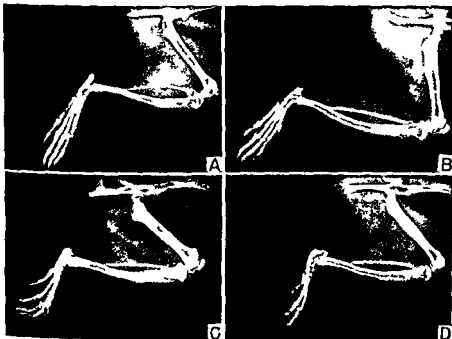


Fig 10 Roentgenograms of the ankle joint of hypophysectomized female rats (× 0 75) A Control injected with inert protein B C D Growth hormone injected Slight, moderate and severe degrees of deformity



FIG 9 Histological sections through the femur and patella of hypophysectomized female rats (H & E × 12) A Control injected with inert protein B Growth hormone injected Increase in size of patella with indefinite and irregular outline of all surfaces and borders

tomized rat in which the roentgenogram had disclosed moderate deformity The plane of section is parasagittal to show the contour of the medial condyles The irregularly thickened cartilage on the articular surface of the joint in the treated rat contrasts with the thin cartilage in the control The bone of the tibial and femoral epiphyses underlying the cartilage shows some irregularity of outline when compared with the control The subpatellar fat pad has become fibrous and more vascular The synovial lining is slightly more evident due to increased fiber content In the sagittal section it could be seen in addition that the cruciate ligaments were thickened and irregularly ossified at their attachments to the tibia and femur On the ven

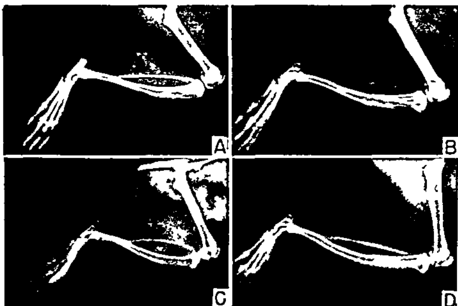


FIG 12 Roentgenograms showing opacities at the ankle joints of normal (left) and hypophysectomized (right) adult female rats (× 0 75) A Normal control injected with inert protein small opacity proximal to calcaneus B Normal rat injected with growth hormone two long opacities near calcaneus C Hypophysectomized control injected with inert protein no opacities D Hypophysectomized rat injected with growth hormone multiple opacities near calcaneus and tibia

cancellous bone extend almost at right angles from the original surface of the bone A squared-off' deformity is thus created which resembles the similar malformation described in metacarpals and phalanges of human acromegalics An abundance of osteoblasts was found in the deep layer of the periosteum The occasional presence of an erosion in the hypertrophic cartilage at the ankle joint is also visible in Figure 11

In Figure 12 is illustrated the second type of abnormality which occurred around the ankle joint In the roentgenograms were seen opacities in areas occupied by ligaments and tendons of this joint They were infrequent in hypophysectomized controls but all normal controls showed at least a small shadow All growth hormone treated rats hypophysectomized as well as intact developed these opacities which were longer and more numerous in rats which possessed a pituitary This incidence was in marked contrast to that of the other changes at the knee and ankle In Table 4 are given the incidence and degree of these changes as observed in roentgenograms

There was great variability in the histologic appearance of these regions of roentgenographic opacity as shown in Fig 13 They ranged from being presumably calcifications in the basophilic areas of otherwise normal dense and regular connective tissue of tendons or fascia to being true bony masses

The incidence and degree of the deformities of the bony structures comprising the ankle joint are shown in Table 3 As with the knee joint none of the uninjected rats hypophysectomized or normal, developed these deformities The ankle joints of intact rats receiving the growth hormone had almost no deformities, whereas those of nearly all of the hypophysectomized injected rats were deformed

Table 3

DEFORMITIES OF THE ANKLE JOINT

Groups	Number of Rats	Degree of Deformity			
		None	*Slight*	*Moderate*	*Marked*
NORMAL					
Controls	15	15	0	0	0
Growth Hormone	15	14	1	0	0
HYPOPHYSECTOMIZED					
Controls	10	10	0	0	0
Growth Hormone	13	1	6	4	2

The exuberant bony growth sometimes present at the distal end of the tibia and fibula is seen in the histological section in Figure 11 Masses of



FIG 11 Histological sections of the distal epiphyses of the tibia and fibula of two hypophysectomized rats treated with growth hormone Erosion of articular cartilage and excessive growth of cancellous bone (H & E × 7 2)

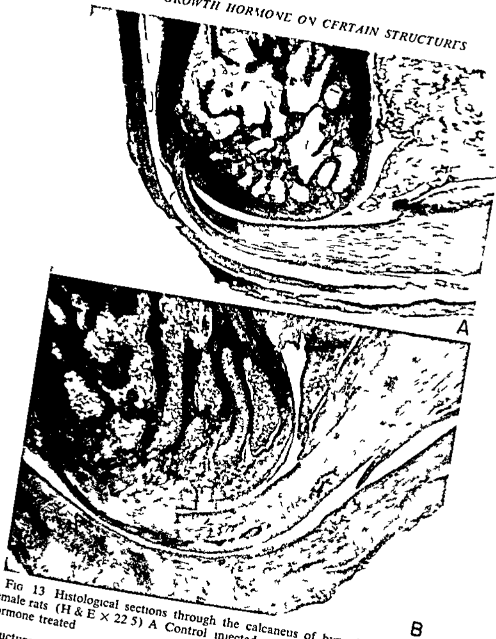


FIG 13 Histological sections through the calcaneus of hypophysectomized female rats (H & E × 22 5) A Control injected with inert protein B Growth hormone treated

structure and relationships had taken place Among these changes were exostoses occasional ankyloses between vertebrae diffuse shadows in the neighborhood of the joints and variability in density of bones which included some rarefactions These alterations were not all constantly present in the injected rats and furthermore they were found occasionally in the old normal controls

One such ossicle lies in the deep crural fascia (just before its descent around the calcaneus to become the plantar fascia), a bursa is present deep to it, which is lined with synovial membrane and which has a cartilaginous transformation on the deep (bursal) surface of the ossicle The bony mass in the tendo calcaneus is not easily distinguished from the surrounding collagenous fibers

Table 4

ECTOPIC BONE AND CALCIFICATION ADJACENT TO ANKLE JOINT

Groups	*Number of Rats*	*Extent of Deposits*			
		None	*Slight*	*Moderate*	*Marked*
NORMAL					
Controls	15	0	10	5	0
Growth Hormone	15	0	1	6	0
HYPOPHYSECTOMIZED					
Controls	10	6	4	0	0
Growth Hormone	13	1	2	6	4

In Figure 13 are also shown the size and density of the bony structure in the os calcis of a hypophysectomized rat receiving growth hormone Such massive deposits of bony tissue occurred within all the small bones of the ankle which led in some instances to almost complete solidification Active bone formation was still occurring as judged by the many osteoblasts being aligned along bony surfaces The loss of fat in the synovial pads and their increased fibrous character was pronounced In a few instances there was evidence of some chronic inflammation of a non specific type in the fibrous tissue and muscle adjacent to the ankle joint It is to be noted that the ankle was the only joint in which inflammation was recorded during the life of the animals

The major deformities were localized at either end of the tibia Other joints of the appendicular skeleton were occasionally found to be slightly deformed but the occurrence was sporadic and the degree was never marked In no case had the joint cavity disappeared in fact an increase of approximately 50 per cent in the width of the joint space was demonstrated by measurements from roentgenograms made in both groups of injected animals at the knee and metacarpo phalangeal joints

Changes in the vertebral column became obvious during the life of the animals Limitations of movement curvatures and fixation in the direction of kyphosis especially in the lumbar region were noted in 10 of the 14 treated hypophysectomized rats Though the deformities of the spine were not observed in all rats during life difficulty in straightening them for dissection was encountered in all It was immediately evident from examination of roentgenograms of the vertebral column that many changes in bony

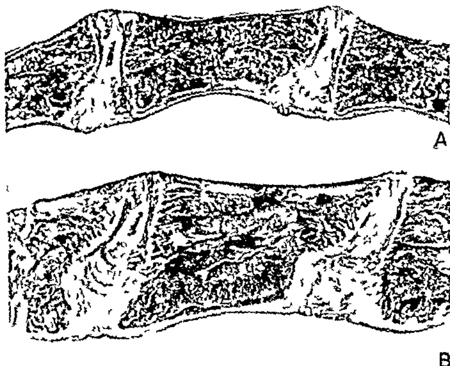


FIG 14 Histological sections through the 9th thoracic vertebra of hypophysectomized female rats (H & E × 12) A Control injected with inert protein B Growth hormone treated Expansion of the ossification center has failed to keep pace with ventral periosteal growth Fibrous tissue fills the resultant gap

increased above that of normal controls the measurements were usually within the limits of normal On the other hand the vertebral dimensions were excessive in almost all of the treated hypophysectomized rats

In Figure 14 are shown the histological appearance of the vertebral bodies and the structures in the so-called intervertebral space which is seen in the roentgenograms The epiphyseal plates of the vertebrae were present and active which accounted for the increased length of the vertebral column However the flattened epiphyseal centers of ossification failed to expand dorso ventrally in pace with the periosteal growth on the ventral aspect of the vertebral body The gap between bones is filled with fibrous connective tissue the arrangement of which is somewhat like that in an intervertebral disc though not so orderly The position of the nucleus pulposus acts as a convenient guide in locating the original center of the intervertebral disc thus serving as a landmark in determining the extent of ventral overgrowth Where the thickened periosteum is continuous with the intervertebral disc there is a fibro-cartilaginous transitional area An excess of osseous tissue from the ends of the vertebral bodies has extended into and around this

One change was found to characterize almost all of the injected animals and it was virtually pathognomonic This was a marked alteration in the shape of the spaces between the bodies of adjacent vertebrae The space widened markedly toward the ventral aspect of the vertebrae, creating the impression of a notch in the roentgenographic image This change could be found in cervical thoracic and lumbar areas and in most affected rats it appeared in the majority of the intervertebral spaces As was shown in the *sections it resulted from an excessive growth of the ventral aspect* of the bodies of the vertebrae The vertebral epiphyseal ossification centers did not expand dorso ventrally at a rate comparable with this periosteal growth but retained their former dimensions Instead of bone proliferating fibrous tissue and fibro cartilage filled the area ventral to the epiphysis creating the impression of a notch in the roentgenogram

Since there was some slight regional variation in the extent of the changes in vertebrae an easily identified region of special interest was chosen for analysis of incidence The junctional area between the lower thoracic and upper lumbar regions has mechanical significance in quadrupeds as indicated by the change in the inclination of the vertebral spines The neutral vertebra at the clinal anticlinal junction is most frequently the ninth thoracic

Table 5

GROWTH AND DEFORMITIES IN VERTEBRAL COLUMN

Groups	*Number of Rats*	*A P Diameter of T 9** *(mm)*	*Vertebral and Intervertebral Deformities†*		
			None	*Moderate*	*Marked*
NORMAL					
Controls	13	1 0 ± 03	13	0	0
Growth Hormone	15	2 2 ± 08	11	4	0
HYPOPHYSECTOMIZED					
Controls	11	1 0 ± 04	11	0	0
Growth Hormone	13	2 8 ± 11	1	2	10

* Neutral vertebra at clinal anticlinal junction
† *Lower thoracic region*

In Table 5 is presented the incidence of roentgenographic alterations at the level of the ninth thoracic vertebra None of the normal or hypophysectomized controls showed deformed intervertebral spaces Some of the intact, injected rats showed moderate deformities though in none were they marked whereas changes occurred in all but one of the hypophysectomized injected rats in which most were extreme The excessive dorso-ventral thickness of the vertebral body can be seen from the measurements made from roentgenograms and given in the table Though the average dorso ventral diameter in some of the intact injected rats was slightly

Table 6

BONE AND JOINT CHANGES INDUCED IN THE RAT BY GROWTH HORMONE COMPARED WITH ACROMEGALY

Criteria for Comparison	*Rat*	*Man*
PROCESSES		
Bone	Periosteal and endosteal overgrowth osteoblasts active bone spongy vascular exostoses	Similar
Cartilage	Articular hyperplasia with localized erosions	Similar
Soft Tissue of Joints	Capsules thickened synovial membranes and fat pads fibrous	Similar
	Inflammatory changes rare ankles only	Variable (secondary to exostoses)
Junctional Areas	Excess growth of bone cartilage fibrous tissue e g tibial tuberosity patella vertebrae (lipping")	Similar
DISTRIBUTION		
Skull	Changes in contour thick bony tables and diploe	Acra enlarged
Mandible	Antero-displacement malocclusion condylar overgrowth	Prognathism[16]
Phalanges	No marked changes	Ends tufted [8] squared porous
Knee and Ankle	Irregular bony growth exostoses (brush like outgrowths of porous bone)	Similar
Vertebrae	Exostoses ankyloses kyphosis	Similar
	Excess ventral periosteal growth increased diameter	Similar
	Intervertebral disc enlargement with protrusion	Similar
Thorax	Bell shaped" (flared ribs everted xiphoid) [6]	Barrel chest
Sesamoids	Increased number knee and ankle	Similar digital tendons
Patella	Exostoses	Similar[20]
Joint Spaces	Increased separation of bone ends due to hypertrophy of articular cartilage	Similar
INCIDENCE	Variable in frequency and degree	Similar
	More common in absence of pituitary	Varies in active and burned out cases
	More common in hind limbs than fore limbs	Similar

tissue in an irregular fashion and in much the same way as has been described elsewhere in comparable junctional areas between fibrous tissue cartilage and bone Occasionally such bony ledges spanned the gap between vertebral bodies

From the wedge shaped intervertebral spaces seen roentgenographically it is easy to visualize how the vertebral column could become distorted into a kyphotic curvature and the vertebrae could become ankylosed by extension of the bony outgrowths into the intervertebral connective tissue

Discussion

There are many striking similarities between the bone and joint changes induced in the rat by chronic administration of growth hormone and those found in the human acromegalic [18] These are presented in Table 6 The above mentioned changes in the vertebrae of rats with experimental gigantism are in many respects comparable to the changes in the vertebrae of human acromegalics The overgrowth of the ventral and lateral surfaces of the vertebrae was recognized by Cushing and Davidoff,[21] and analyzed by Erdheim [2, 3] An illustration from the former source is reproduced in Figure 15 The fungus like character of the outgrowth of porous bone from the ventral surface of the vertebrae is not, however the characteristic change seen in the rat Here the excessive ventral periosteal bone is usually *reorganized and incorporated so that only a faint roentgenographic shadow* remains to mark the boundary between the old and the added new bone Exuberant outgrowths of bone were seen at the malleoli of the tibia and fibula of the injected rats These were comparable to the overgrowths seen on the ventral surfaces of the vertebral bodies and at the tips of the ungual phalanges of acromegalic patients

Most of the excessive growth and abnormalities in bone and joints described in man as in the rat can be interpreted as due to over stimulation by growth hormone of periosteal bone which continues even after normal epiphyseal closure Erdheim and others assumed that in areas where cartilage persists as in the mandibular condyle costochondral junction and intervertebral fibrocartilage there is in acromegalics a re-establishment of *endochondral ossification New bone formation in rats at so-called* junctional areas where the transformation of periosteal fibrous tissue into cartilage occurs may also be considered as endochondral ossification In respect to *the long bones of the rat there is no need for assuming that new* cartilage formation is obligatory for excessive bone formation at buttress areas although it can occur as an adjunct to deformity as at the knee where the epiphyseal cartilage plates of femur and tibia remain The excessive bone formation at the ankle where resorption of the epiphyseal plates had taken place before the onset of growth hormone administration resulted primarily from periosteal stimulation even though metaplasia was present in fibrous tissue

In contrast to the findings in human acromegaly erosions of articular cartilage in the rat ankle joint were not conspicuous although it is true that irregularities in the width of the hypertrophic articular cartilages were characteristic No atrophy extensive enough to give roentgenographic evidence of rarefaction was noted in the trabeculae at the ends of the bones Although the paws of growth hormone treated rats were thickened their length did not exceed normal and no tufting of the phalanges was noted An increase in the number of sesamoid bones was found more frequently in rats than has been reported for human acromegalics

Many of the skull changes in rats are difficult to interpret in terms of the cranial mandibular and dental changes characteristic of human acromegaly Differences in the degree of development of the nasal accessory sinus system and in the mechanics of mastication and the lack of a chin in the rat disguise basic similarities in the acromegalic process

The thoracic deformities in man and rat are essentially attributable to a reawakened rib growth with attendant lengthening of the ribs and deepening of the thorax Both show the enlarged costochondral junctions due to the hypertrophic cartilage and the advancing endochondral and periosteal bone formation

Like the acromegalic changes in man the growth hormone induced changes in rats are not limited to the stimulation of excessive growth in selected regions articular cartilage *for example* is hypertrophied in both species Many changes occur in the connective tissues and those pertaining particularly to the joints include the fibrous replacement of fat pads thickening of the capsule and overgrowth at the junctional areas between periosteum cartilage and bone

Whereas the osteoarthropathies in rats resulting from prolonged treatment with growth hormone have many points of resemblance to human acromegalic arthropathy they have little in common with other polyarthritic joint disorders In these rats very few features were found which characterize rheumatoid arthritis such as inflammatory changes within and around the joints synovial adhesions obliteration of the joint cavity and atrophy of trabecular bone Similarly signs of degenerative osteoarthritis were lacking such as atrophy of articular cartilage denudation and eburnation of the condyles and joint mice In some instances old age in both rat and man undoubtedly affects the picture while inflammatory changes particularly those secondary to exostoses and weight bearing are probably superimposed in others

Summary

In addition to the anticipated gigantism multiple bone and joint abnormalities occurred in rats injected chronically with growth hormone The changes were more numerous and more severe in the hypophysectomized injected rats than in intact rats similarly treated Only a few abnormalities



FIG 15 Ventral bony overgrowth in the eleventh thoracic vertebra from a case of human acromegaly (below) as compared with the normal (above) After Cushing and Davidoff Rockefeller Institute for Medical Research Monographs No 22 1927 Fig 83

The localization of finer details of the abnormalities in the two species may be expected to differ because of inherent biologic differences e g orthograde versus pronograde posture and the persistence in rats of some epiphyses even in aged animals The bone and joint changes reported in human acromegalics probably will always be somewhat variable inasmuch as some cases are studied while still actively progressive and others are inactive or burned out acromegalics On the other hand in rats it was obvious from the size and the number of osteoblasts present that growth was still continuing even after prolonged treatment

In the growth hormone injected groups of rats the chief source of variability was the presence or absence of the pituitary The changes were always more severe in the hypophysectomized rats The relatively rapid appearance of changes in the knee and ankle noted by Reinhardt and Li[7] in adrenalectomized gonadectomized rats under the influence of growth hormone and the greater number of changes described here as occurring in the hypophysectomized rat as contrasted with the intact rat point to the importance of the hormonal balance

Connective tissue filled the space between the vertebrae ventral to the epiphyses

Periosteal growth covering the widened intervertebral disc sometimes caused ankylosis

Counterparts are found in human acromegaly for the majority of the proliferative processes here described and for the gross deformities which result

References

1 Evans H M and J A Long *Anat Record* **21** 62 (1921)
2 Evans H M and J A Long *Anat Record* **23** 19 (1922)
3 Putnam T H Benedict E B and H M Teel *Arch Surg* **18** 1708 (1929)
4 Evans H M Simpson M E Meyer K and F L Reichert *Memoirs U of C* **11** 421 (1933)
5 Silberberg M and R Silberberg *Arch Pathol* **36** 512 (1943)
6 Selye H *First Annual Report on Stress* Montreal Acta Inc 1951 244
7 Reinhardt W O and C H Li *Science* **117** 295 (1953)
8 Simpson M E Asling C W and H M Evans *Yale J Biol and Med* **23** 1 (1950)
9 Evans H M Becks H Asling C W Simpson M E and C H Li *Growth* **12** 43 (1948)
10 Becks G Collins D A Asling C W Simpson M E Li C H and H M Evans *Growth* **12** 55 (1948)
11 Evans H M Simpson M E and C H Li *Growth* **12** 15 (1948)
12 Simpson M E Evans H M and C H Li *Growth* **13** 151 (1949)
13 Moon H D Simpson M E Li C H and H M Evans *Cancer Research* **10** 297 (1950)
14 Moon H D Simpson M E Li C H and H M Evans *Cancer Research* **11** 535 (1951)
15 Collins D A Becks H Asling C W Simpson M E and H M Evans *Growth* **13** 207 (1949)
16 Weinman J P and H Sicher *Bone and Bones* St Louis The C V Mosby Company 1947
17 Becks H Asling C W Simpson M E Li C H and H M Evans *Growth* **13** 175 (1949)
18 Kellgren J H Ball J and C K Tutton *Quart J Med* **21** 405 (1952)
19 Evans H M Asling C W Simpson M E and H Becks *Growth* **13** 191 (1949)
20 Waine H Bennett C A and W Bauer *Am J Med Sci* **209** 671 (1945)
21 Cushing H and L M Davidoff *Rockefeller Inst for Med Research Monographs* **22** 1 (1927)
22 Erdheim J *Virchow's Arch pathol Anat u Physiol* **28** 197 (1931)
23 Erdheim J *Pathologie und Klinik in Einzeldarstellungen* Vol 3 Berlin Springer Verlag OHG 1931

of the types noted in experimental groups were seen in their respective controls

1 Excessive proliferation of osseous, cartilaginous and fibrous tissues led to the gross changes

(a) Periosteal bony overgrowth led to thickened enlarged and deformed bones to exaggerated osseous attachment sites of ligaments and tendons and to exostoses

(b) Excessive chondrogenesis in articular cartilage led to increased separation of bones at joints in regions where there was attendant endochondral osteogenesis (such as at epiphyseal discs mandibular condyle and the patella) it led to increased bone

(c) Excessive production of fibrous connective tissue led to fibrosis of intra and periarticular structures such as fibrous replacement of fat pads thickening of joint capsules and periosteum and enlargement of tendons and ligaments

(d) Metaplastic transformations also occurred in fibrous tissue leading to bone or cartilage development in abnormal places such as ectopic nodules in ligaments and tendons

(e) At junctional regions between bone cartilage and periosteum (as in the so called buttress areas of long bones) where overproduction of more than one of these tissues occurred the disturbed balance resulted in such gross changes as overhanging epiphyses and lipping These deformities included excessive periosteal bone formation with adjacent defects in endochondral bone formation due to deficient expansion of the epiphyseal disc and replacement fibrosis

2 The roentgenographic survey showed the majority of these deformities to be located in the hind limb (knee and ankle) and in the vertebral column the other joints being spared or inconstantly affected

(a) At the knee the femoral and tibial condyles, sesamoid bones and menisci were enlarged and irregular

The epiphyses showed malformation and lipping with rarefaction *of the cortex of the neighboring shaft*

The joint space was increased

(b) At the ankle overproduction of bones and malformation was found The malleoli of the tibia and fibula were squared and the calcaneus was deformed Ectopic calcifications and sesamoid bones appeared in the tendons and ligaments behind the joint

(c) In the vertebral column limitation of movement and kyphosis had been evident during life

The dorso-ventral diameter of the vertebral bodies had increased by periosteal osteogenesis there was no accompanying increase in the epiphyses

sectomized animal or raises them above normal in the intact animal Growth hormone preparations will do this[3, 13] although preparations of equal growth promoting activity show great variability in their renal effects The information gained by this second type of procedure cannot be any more definite than the separation and purification of the various anterior lobe hormones

We may first consider some observations with the first type of procedure After thyroidectomy there are slight falls in inulin and in Diodrast® (D) clearance and in TmD but these are much less than after hypophysectomy The values are restored essentially to normal with dessicated thyroid 0 1 g /kg /day orally Bilateral ovariectomy has no effect on these clearances or on TmD The unsupported adrenalectomized dog of course shows great falls in these renal functions but it was concluded that the falls in renal function seen after hypophysectomy are not due to the resultant depression of adrenal cortical function First DOCA support adequate to maintain the renal function of adrenalectomized dogs at or near normal has no protective effect on the renal function of hypophysectomized dogs Second our ACTH preparations had no effect on the renal functions of hypophysectomized dogs ascribable to adrenocortical stimulation the occasional enhancing effect of one ACTH preparation in hypophysectomized dogs was also seen in adrenalectomized dogs and must therefore be ascribed to some effect other than adrenocortical stimulation[14] The conclusion was therefore reached that the major part of the effects of hypophysectomy in depressing the clearance and Tm values is due to the loss of some anterior lobe principle(s) other than the thyrotropic gonadotropic or adrenotropic although loss of thyrotropin undoubtedly plays a part

We may begin our consideration of the observations carried out through the second type of procedure with those on growth hormone administration[1, 13] When 0 5 mg /kg daily of growth hormone preparation 3PKR3 generously supplied by Dr I M Bunding of the Armour Research Laboratories was given subcutaneously to normal dogs great increases in inulin and PAH clearances and in TmPAH were seen This effect has not appeared in 5 days but is present in 9 or 10 days Similar effects were obtained with Armour s preparation 22KR2

When the same dose of either of these preparations was given to hypophysectomized dogs even greater percentage increases were obtained and the effects were manifested earlier

The question of whether these effects might be due to the presence of thyrotropin or ACTH must be considered While thyrotropin in sufficient doses as well as dessicated thyroid substance or thyroxin will raise the renal functions under discussion the amounts of thyrotropin present in our doses of these growth hormone preparations were considerably less than the minimal amounts of thyrotropin required to produce detectable effects on renal function The daily doses of 0 5 mg /kg of 3PKR3 and 22KR2

10

Growth Hormone and Renal Function

H L White

Department of Physiology Washington University School of Medicine St Louis

It is well established that removal of the adenohypophysis or depression of its function in the dog rat or man is followed by diminution in renal plasma flow (RPF) as measured by diodrast or para aminohippurate (PAH) clearance or by Diodrast® clearance/Diodrast® extraction in urea clearance in glomerular filtration rate (GFR) as measured by insulin or creatinine clearance and in Diodrast® PAH and glucose Tm [1, 3 4 5 6 7] A water load is excreted more slowly [3 6] Ability to conserve sodium on a restricted intake has been reported as essentially normal [3] and as slightly but measurably impaired [8] Rate of excretion of a salt load is unchanged after hypophysectomy in the dog [9] Ammonia excretion in response to acidosis is unchanged by hypophysectomy [10] as is renal glutaminase activity [11] In spite of chronically reduced renal blood flow and filtration rate plasma levels of urea nonprotein nitrogen sodium potassium and chloride remain within normal limits with animals on reasonably normal diets Diminished urea output is a reflection of diminished protein intake blood urea levels although still within normal limits being somewhat higher than those for normal dogs at comparably reduced protein intakes

The question arises as to what is the anterior lobe principle(s) whose loss is responsible for the changes in renal function following hypophysectomy Two types of procedure are available The first is to remove various target organs from otherwise intact animals and see whether renal functional changes are produced comparable with those seen after hypophysectomy Thus if changes were seen after castration similar to those occurring after hypophysectomy one might conclude that a major part of the hypophysectomy effect is due to lack of gonadotropic hormones The other type of procedure is to give various anterior lobe hormone preparations and see which of these restores to normal the depressed renal functions in the hypophy

Table 2

EFFECTS OF GROWTH HORMONE ON RENAL FUNCTIONS IN HYPOPHYSECTOMIZED DOGS

		PAH Clearance	*Inulin Clearance*	*PAH Tm*	*Plasma NPN*	*Plasma Glucose*
K39						
		cc/min/M²	cc/min/M²	mg/min/M²	mg %	mg %
6/13/47	Normal	264	95	19		
6/16/47	Normal	263	96	23		
6/20/47	Simple hypophysectomy					
7/17/47		158	63	6 1		
9/10/47		176	44	8 8		
12/31/47		151	45	8 6		66
2/16/48		129	52	5 6	37	71
11/22/48		124	51	6 8		65
11/24/48	through 12/3 48—0 5 mg kg growth hormone (3PKR3)					
11/29/48	5 days of growth hormone	235	82	17	28	106
12/3/48	9 days of growth hormone	261	95	18		99
K42						
11/10/47	Normal	247	77	22		95
12/15/47	Normal	276	95	26		94
1/21/48	Simple hypophysectomy					
2/26/48		130	53	5 1	66	92
3/24/48		124	49	6 5	50	80
12/7/48		149	54	9 3		82
12/7/48	through 12/16/48—0 5 mg/kg growth hormone (3PKR3)					
12/16/48	9 days of growth hormone	353	110	26	27	121

Growth hormone daily subcutaneously (Courtesy *Am J Physiol* 157 49 1949)

Table 1

EFFECTS OF GROWTH HORMONE ON RENAL FUNCTIONS IN NORMAL DOGS

	PAH Clearance	*Inulin Clearance*	*PAH Tm*	*Plasma Glucose*
Dog K43				
	cc /min / M²	*cc /min / M²*	*mg / min /M²*	*mg %*
10/28/48 Normal	352	116	20	81
10/29/48 through 11/10/48—0 5 mg /kg growth hormone (3PKR3)				
11/3/48 5 days of growth hormone	336	117	27	81
11/10/48 12 days of growth hormone	849	170	33	91
11/12/48	608	138	20	79
11/16/48	308	91	16	80
Dog K44				
12/21/48 Normal	229	78	19	93
12/21/48 through 12/27/48—0 5 mg /kg growth hormone (3PKR3)				
12/28/48 through 12/30/48—1 25 mg /kg growth hormone (3PKS3R)				
12/30/48 9 days of growth hormone	388	124	35	117

Growth hormone daily subcutaneously (Courtesy *Am J Physiol* 157 48 1949)

stance not yet separated from it It is assumed that the renotropic activity is more easily destroyed than the growth promoting activity in procedures designed to separate growth hormone

Earle de Bodo et al[5] reported even greater falls in clearances after hypophysectomy than had been found by White Heinbecker and Rolf and found that growth hormone administration increased the depressed clearances the daily water intake and the rate of elimination of a water load in hypophysectomized dogs[1] although clearance values were not restored completely to normal They later reported[16] that administration of ACTH or of cortisone or hydrocortisone also raised the depressed clearance values of hypophysectomized dogs although not to normal and concluded that the adrenocortical regression resulting from loss of ACTH is one of the factors responsible for the marked reduction in certain renal functions of hypophysectomized dogs

Why hypophysectomy with the New York investigators resulted in so much greater percentage reductions in clearances than with us is not clear our animals were proved by serial sections to have complete loss of adenohypophysis Their finding of increases in function on giving ACTH as compared with our usual failure to get such increase may be related to their lower levels obtaining before ACTH to the somewhat larger doses employed or to their longer periods of administration They do not report on whether DOCA improves these functions after hypophysectomy

Boss Osborn and Renzi[4] found that adrenal cortical extract administration to hypophysectomized rats did not raise the lowered PAH clearance It had no effect on GFR 20 or more days after hypophysectomy but produced slight rises before the twentieth day It also increased the rate of water sodium and potassium excretion

In a patient with diabetes insipidus and anterior lobe hypofunction growth hormone had no significant effect on inulin or PAH clearances while ACTH caused about a 50 per cent rise in inulin but no significant change in PAH clearance

Very large doses (400 to 600 mg daily) of ACTH to normal human subjects raised inulin and PAH clearances about 30 per cent while large doses of cortisone (100 to 200 mg daily) produced no significant change In cases of Addison s disease probably significant increases in inulin but not in PAH clearance were produced by cortisone[17]

Hypophysectomy brought about large falls in inulin and PAH clearances in previously adrenalectomized DOCA supported dogs Growth hormone produced large increases in inulin and PAH clearances in adrenalectomized DOCA-supported dogs Only a part of the effect could be accounted for by the contaminant thyrotropin an insignificant fraction with one preparation and about half with the other It was concluded that in these dogs the drops in clearances following hypophysectomy were not related to loss of ACTH and that loss of both thyrotropin and growth hormone played a part[18]

were assayed to have thyrotropic activity equivalent to 90 and 15 gamma respectively of Armour s standard thyrotropin It was found that 130 gamma/kg daily of Armour s standard thyrotropin had only a slight or no effect on clearances and none on oxygen consumption in 3 normal dogs[13] This is about 50 per cent more than and 9 times as much as the thyrotropin contents of our doses of 3PKR3 and 22KR3 respectively

Contamination with ACTH is believed not to be responsible for the renal effects of our growth hormone preparations First the amounts of ACTH were negligible there being less than the equivalent of 0 02 mg standard ACTH per mg of growth hormone Second, in only 1 of 5 experiments on 3 hypophysectomized dogs given rather large doses of ACTH was a certain increase in renal functions seen with a questionable increase in one other There was no effect with 1 normal dog In 1 of 3 experiments on an adrenalectomized dog however a significant increase was also seen indicating that the response when present in hypophysectomized dogs need not be ascribed to adrenocortical stimulation

Difficulties in the interpretation that the anterior lobe principle responsible for the above effects is growth hormone arose when some additional growth hormone preparations while active in promoting growth had no effect on renal function in normal dogs although still effective in hypophysectomized dogs It is clear that the hypophysectomized dog is a more sensitive subject whatever the nature of the active principle

Three possible explanations of the lower renotropic/growth promoting ratio of certain growth hormone preparations were considered First that the activity is not due to growth hormone but to a renotropically active contaminant (not thyrotropin or ACTH) present in larger amount in the more active preparations Second that the low activity of the less active preparations is due to a substance which inhibits renal effects without inhibiting growth effects the inhibitor contaminant being present in the less active and not in the more active preparations Third that the mother molecule of growth hormone may be differentially damaged in such a way that the renotropic activity is impaired while growth promoting activity is retained this change being more marked in the less active batches

In an attempt to get information on the first two possibilities various fractionation products low in growth promoting activity and kindly supplied by Dr A E Wilhelmi were tested No renotropically active contaminant was found in any of the fractions tested Furthermore none of these fractions had an inhibitory effect when added to known renotropically active preparations Since no evidence was obtained for either of the first two possibilities of the preceding paragraph the third is tentatively accepted

We may summarize our views at this stage by stating that a major part of the depression of certain renal functions after hypophysectomy is due to the loss of some anterior lobe principle(s) other than gonadotropin thyrotropin or ACTH This is probably growth hormone or some sub-

theirs while destruction of pitressin forming cells was achieved in both cases

Growth hormone effects on electrolyte excretion may be referred to briefly It has already been mentioned that one group has found the ability of hypophysectomized dogs to conserve sodium on a low salt intake to be essentially normal [2] while another group has found some impairment [8] Growth hormone administration produced transitory retention of sodium and chloride in normal rats and in adrenalectomized rats with or without adrenocortical extract support Potassium and nitrogen retentions were maintained throughout the periods of growth hormone injections Since these retentions occurred in adrenalectomized rats it was concluded that these actions of growth hormone are not dependent on a release of adrenocortical hormones but are probably due to a direct effect of growth hormone on the peripheral tissues or on the tubular cells [1]

On reasonably normal diets dogs remain in sodium balance and show normal plasma sodium levels both with long lasting decreases of glomerular filtration rate induced by hypophysectomy and with long lasting increases induced by growth hormone administration These findings show that essentially normal tubular adaptations to changes in filtered load of sodium can still occur

Various workers have shown that growth hormone administration increases total body water in normal and hypophysectomized rats and in normal dogs and cats The thiocyanate space in normal and hypophysectomized rats and plasma volume in dogs is increased by growth hormone Total body water (antipyrine space) extracellular water (inulin or thiocyanate space) and total exchangeable sodium per kilogram body weight or per square meter are higher in acromegalics than in normal human subjects [3]

References

1 White H L and P Heinbecker *Amer J Physiol* 130 464 (1940)
2 White H L Heinbecker P and D Rolf *Am J Physiol* 149 404 (1947)
3 Earle D P Jr deBodo R C Schwartz I L Farber S J Kurtz, M and J Greenberg *Proc Soc Exp Biol Med* 76 608 (1951)
4 Boss W R Osborn C M and A A Renzi *Endocrinology* 51 66 (1952)
5 Leaf A Mamby A R Rasmussen H J P Marasco *J Clin Investigation* 31 914 (1952)
6 Beaumont G E and I D Robertson *Brit Med J* 2 356 (1943)
7 Luft R and B Sjogren *Acta Endocrinologica* 4 351 (1950)
8 Rolf D Surtshin A and H L White *Am J Physiol* 169 576 (1952)
9 Martin H F and H L White *Am J Physiol* 170 532 (1952)
10 Hartmann A F Jr Harris F D Martin H F Rolf D and H L White *Am J Physiol* 167 563 (1951)
11 White H L and D Rolf *Am J Physiol* 174 27 (1953)
12 White H L Heinbecker P and D Rolf *Am J Physiol* 157 47 (1949)
13 White H L Heinbecker P and D Rolf *Am J Physiol* 165 442 (1951)

There is a unanimity of opinion among different investigators that hypophysectomy produces large falls in inulin and PAH or D clearances and in Tm and that a part of this effect is due to the resultant depression of thyroid activity There is some difference of opinion as to the relative importance of the loss of growth hormone and of ACTH All of our growth hormone preparations have raised oxygen consumption and more we believe than can be accounted for by their thyrotropin content How important a factor is the fall in metabolic rate after hypophysectomy and the rise with hormone administration is not clear Only in the work of Davis and associates[18] has food intake been carefully controlled Since they obtained changes in clearances with no change in food intake it is apparent that the effects cannot be ascribed merely to changes in intake resulting from lack or administration of hormones In an attempt to make a statement acceptable to most workers in the field we believe it is fair to say that the falls in kidney function observed after hypophysectomy can be explained only in part by loss of thyrotropin that loss of gonadotropin plays little or no part that loss of growth hormone or some substance not yet separated from it plays an important part and that loss of ACTH may play some part less important than that of loss of growth hormone

A quite different view has been expressed by Keller and associates[19,20] While there is nothing in their work to contradict the view that loss of anterior lobe depresses inulin and PAH clearances and TmPAH they present the further concept that complete destruction of the neurohypophysis (including rather extensive destruction of the ventral tuberal and ventral anterior hypothalamus) *without* obvious severe adenohypophyseal involvement produces comparable effects in addition to diabetes insipidus They further report that these depressed renal functions can be restored to normal by pitocin but not by pitressin administration Their evidence that anterior lobe involvement is only slight appears convincing It is based on the nature of the operative approach the relatively normal gross and microscopic appearance of the anterior lobe the relatively slight increase in insulin sensitivity and absence of marked sexual regression and adrenal cortical atrophy

These findings were unexpected by us in view of our finding that anterior hypothalamic lesions producing permanent and maximal diabetes insipidus through complete destruction of the supraoptic and partial destruction of the paraventricular pathways and nuclei resulted in only slight and transitory falls in renal function which were ascribed to moderate and reversible damage to the anterior lobe We can only conclude that their lesions produced more extensive damage in the anterior hypothalamic areas critical for maintenance of renal function than did ours In order to reconcile these sets of findings one must assume that pitocin forming cells in the ventral anterior hypothalamus escaped destruction with our lesions and not with

We have repeated these studies and have obtained similar results In the bone of the untreated animals there is a general distribution of calcium (Ca^{45}) with no evidence of concentration in the epiphyseal region which is the type of picture obtained with very mature bone In the bone auto radiographs of the animals which received growth hormone one finds a very heavy line of Ca^{45} uptake in the region of the growing epiphyses and along the periosteum This would indicate that new bone salt was being laid down in these areas as indeed was evident in the histological sec tions

The experiment was repeated in young animals which were hypophysec tomized for 3 weeks and then given 12 mg of growth hormone during the ensuing 3 weeks From the bones of the intact normal animals both un treated and treated with growth hormone autoradiographs demonstrated heavy lines of Ca^{45} deposition in the area of epiphyseal growth In the bone of the untreated hypophysectomized animal a small amount of Ca^{45} was observed in the epiphyseal region but not nearly as much as was found in the growing epiphyses of the growth hormone treated animals From these experiments it is evident that growth hormone causes restoration not only of normal cartilage growth but also of the active deposition of calcium in that region

We have carried out further experiments of a preliminary nature on the effects of growth hormone on animals suffering from severe phosphorus deficiency and I would like to say just a word or two about these They were carried out with Freeke Van Houhuys Kohl with the cooperation from the Institute of Experimental Biology University of California

Now phosphate is as important for the growth of soft tissue as is pro tein When an animal is deprived of dietary phosphate that which is neces sary for growth of tissue is obtained from the skeleton through an active resorption of bone The excess bone calcium is excreted in the urine and the animals develop severe osteoporosis In addition they develop advanced rickets because of the low level of phosphate and the severity of the rickets depends on the degree of growth which is obtained In this experiment we restricted weanling rats of 3 weeks to a diet which was practically free of phosphate i e *it contained 50 parts per million The phosphorus deficient* animal consumed only about one half as much food as the ordinary controls so that it was necessary to restrict the pair fed controls to 6 grams per day of the control diet containing phosphorus Some growth occurred in both groups but it was very limited At the end of 4 weeks on the diet the animals received growth hormone 1 0 mg per day intraperitoneally fol lowing which there was nitrogen retention and an increase of weight In the 10 day period of the experiment the weight gain amounted to 6 grams for the phosphorus deficient animals and to about 14 grams for the pair fed controls An accelerated loss of mineral was observed in the deficient animals and when radioactive strontium was injected into these animals as a

14 White H L Heinbecker P and D Rolf *Am J Physiol* **156** 67 (1949)
15 deBodo R C Schwartz I L Greenberg J Kurtz M Earle D P Jr and S J Farber *Proc Soc Exp Biol Med* **76** 612 (1951)
16 Earle D P Farber S J deBodo R C Kurtz M and W M Sinkoff *Am J Physiol* **173** 189 (1953)
17 Burnett C H *Trans Second Macy Conference on Renal Function* 1951 106
18 Davis J O Howell D S Laqueur G L and E C Peirce II *Am J Physiol* **176** 411 (1954)
19 Handley C A and A D Keller *Am J Physiol* **160** 321 (1950)
20 Demunbrun T W Keller A D Levkoff A H and R M Purser Jr *Report 135 Fort Knox Army Medical Research Lab* January 1954
21 Stein J D Jr Bennett L R Batts A A and C H Li *Am J Physiol* **171** 587 (1952)
22 Surtshin A Rolf D and H L White *Am J Physiol* **165** 429 (1951)
23 Ikkos D Luft R and B Sjogren *J Clin Invest* **33** 989 (1954)

DISCUSSION

Effects of Growth Hormone on Certain Structures

Designated Discussion

D HAROLD COPP (The University of British Columbia, Faculty of Medicine) I feel very humble for being called on to open the discussion of these two excellent papers for I am a novice in the presence of experts and I came here principally to listen and learn I shall not presume to discuss Dr White s paper because there are many in the audience who are far better qualified to do so I will confine my remarks therefore to a consideration of the effect of growth hormone on the skeleton and particularly on the mineral of the skeleton

It is common knowledge that the administration of growth hormone to both intact and hypophysectomized rats will result in a positive calcium and phosphorus balance and in an increased mineral content of the skeleton Parks and Reinhardt in 1942 attempted to demonstrate an effect of growth hormone and hypophysectomy on the uptake of radioactive strontium by the bones of the rat They were unable to find any significant effect and this dampened enthusiasm for such studies with radioactive isotope until almost 9 years later The reason is I think that at the time the experiment was carried out some of the difficulties and problems inherently connected with isotope studies of bones were not fully understood This work was then repeated by Ulrich Reinhardt and Li who used radioactive calcium in 4 month old male rats which had been hypophysectomized 2 months earlier and given growth hormone for 3 weeks prior to the injection of the radioactive calcium They were able to demonstrate that the retention of calcium (Ca^{45}) in the tibia of the treated animals was greater than that of the control group More significant was their demonstration by autoradiographs of a change in the distribution of the newly deposited radioactive calcium

tag for calcium which it resembles biologically the skeleton was unable to retain much of the tagged element As a result the amount of strontium retained in the deficient animals was about one fifth of that found in the normal controls The administration of growth hormone had no significant effect in the control animals but in this short period it appeared to increase the severity of the mineral deficiency in the depleted animals In the x rays of the bones of these animals the control group showed well mineralized skeletons with very narrow epiphyseal plates The x rays of the deficient animals untreated with growth hormone revealed the wide epiphyseal cartilage characteristic of rickets and the low intensity of the mineral in the skeleton In the animals treated with growth hormone the epiphyseal cartilage is considerably wider than in the untreated animals This is shown very clearly in the histological sections in Figure 1 The upper two pictures are the bone sections of the normal pair fed controls in which the epiphyseal cartilage is narrow and about that which you would expect in a hypophysectomized animal There is very little evidence of growth hormone effect perhaps a suggestive widening of the epiphyseal plate but it is not very significant In the sections from the phosphorus-deficient animal not receiving growth hormone shown in the lower left picture the epiphyseal plate is wider as you would expect in an animal developing rickets Following the administration of growth hormone (lower right) however there is a tremendous growth of cartilage and a further increase in the formation of uncalcified osteoid matrix resulting in an epiphyseal plate which in one animal was almost one millimeter thick

These experiments suggest two things Firstly that growth hormone can stimulate cartilage growth even in the presence of a severe dietary deficiency From this observation it would appear likely that the effect of growth hormone is primarily on the growth of cartilage and bone protein and that the increase in mineral content of bone is secondary The second point which I would like to raise is that growth hormone may provide a convenient additional growth stress in the development of nutritional deficiency and as such may prove to have some auxiliary experimental uses

General Discussion

HANS SELYE I would like to ask Dr Simpson two questions Firstly was there any change in the adrenals of these giant animals treated chronically with growth hormone following hypophysectomy? Particularly was there any change in weight? In our experience the adrenal weight did not increase in animals which became quite gigantic under the influence of enormous doses of STH This is not the time to discuss the manner in which STH acts on the adrenal We think it has an effect without producing an actual increase in adrenal weight I would like to know what her experience was

Secondly what is the correlation between the changes she described here

A



CONTROL-UNTREATED

B



CONTROL + GROWTH H



P DEFIC -UNTREATED

A



P DEFIC + GROWTH H

B

Fig 1 Tibial metaphyses of phosphorus deficient and pair fed control rats (A) untreated (B) treated with 1 mg hypophyseal growth *hormone per day for* 9 days (× 45)

injection of some mild irritant such as formalin in doses which normally would not cause much inflammation

Finally in confirmation of Dr Simpson's work I might mention some other observations we have made in collaboration with Dr E Salgado It will bring out also an additional point which has not been mentioned today and which is worth discussing The animals in Figure 2 are all hypophysectomized They have been shaved to show what the skin looks like The animal on the far right is untreated while the animal in the middle has been treated with STH throughout the experiment The animal became gigantic and note that its joints especially the ankle joints are much swollen and the skin is extremely tense in comparison to the control animal The same amount of growth hormone was given to the animal on the left but the hormone was interrupted when the two had reached the same weight The same joint changes are apparent You can see that it lost considerable weight as a result of STH withdrawal at a time when under continued hormone administration growth did not continue Now this so-called resistance to chronic growth hormone treatment is a well known fact If you give large doses of STH the growth curve gradually flattens out I think the type of observation illustrated by Figure 2 definitely proves that there is no actual



Fig 3

and those which have been described in a paper from the same laboratory by Dr Li and others and which appeared in Science? This paper presented some radiological evidence of ossification in the joint region which was interpreted as an arthritis Now, I wonder whether that was just a difference of interpretation or whether there was a difference in the actual findings I realize that it is very difficult to make a clear cut distinction between a purely inflammatory phenomenon and proliferation of connective tissue The end result of chronic inflammation is a scar which though a manifestation of inflammation is not itself accompanied by inflammatory infiltration cells Nevertheless it is a reaction to injury Perhaps the excessive proliferation of connective tissue in regions of local stress, i e , insertion points of tendons etc might be a type of local reaction to the injury of mechanical traction We agree that inflammation of the joints is rare after STH treatment (in the absence of topical injury) but quite in agreement with Dr Simpson if this does occur we find it only in the paw and ankle joints of the hind legs of animals chronically treated with small ascending doses of growth hormone We find it much more frequently in animals given large doses of growth hormone abruptly or in animals receiving any amount of growth hormone in addition to even mild local stress The latter is produced by an

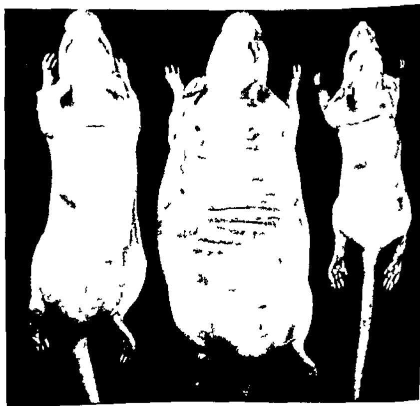


FIG 2

injection of some mild irritant such as formalin in doses which normally would not cause much inflammation

Finally in confirmation of Dr Simpson s work I might mention some other observations we have made in collaboration with Dr E Salgado It will bring out also an additional point which has not been mentioned today and which is worth discussing The animals in Figure 2 are all hypophysectomized They have been shaved to show what the skin looks like The animal on the far right is untreated while the animal in the middle has been treated with STH *throughout the experiment* The animal became gigantic and note that its joints especially the ankle joints are much swollen and the skin is extremely tense in comparison to the control animal The same amount of growth hormone was given to the animal on the left but the hormone was interrupted when the two had reached the same weight The same joint changes are apparent You can see that it lost considerable weight as a result of STH withdrawal at a time when under continued hormone administration growth did not continue Now this so-called resistance to chronic growth hormone treatment is a well known fact If you give large doses of STH the growth curve gradually flattens out I think the type of observation illustrated by Figure 2 definitely proves that there is no actual



Fig 3

resistance to the hormone itself since it can maintain an abnormally large body weight If the hormone is now interrupted the animal still shows the lack of it so the hormone still has some effect

In Figure 3 is shown the result of an experiment which is pertinent to Dr Simpson s findings of the changes induced in joints by growth hormone The animals are held in exactly the same position and both are deeply anesthetized Just as Dr Simpson said the hypophysectomized control on the bottom has a flexible spine and accordingly bends when extended while the hypophysectomized and growth hormone treated animal has a spine so much involved in whatever you call the lesion that it remains rigid when similarly extended

MIRIAM SIMPSON In regard to the first question there was no increase in the size of the adrenals Now as to the comparison of our work with that of Reinhardt and Li it is very interesting that our major findings were in the hypophysectomized animals I described only those changes which occurred chronically while they were concerned with changes which developed rather quickly It is interesting that in their gonadectomized and adrenalectomized animals they observed the lesions which we had seen in our hypophysectomized animals This suggests that a hormonal imbalance is certainly part of the picture We cannot say what earlier changes took place in our animals There was nothing which simulated rheumatism or rheumatoid arthritis and we saw only an occasional inflamed ankle joint No cellular infiltration into the connective tissue about the joints was noted but there was an increased density as a result of a transformation of fat tissue into connective tissue

CHARLES DENKO (University of Chicago) We have studied the effect of growth hormone on cartilage using radioactive sulfur as a tracer We have noted that hypophysectomy causes a decrease in the uptake of radioactive sulfur which we interpret to mean a decrease in the synthesis of chondroitin sulphate We have observed that growth hormone causes a 3 to 4 fold increase in the uptake of sulfur indicating either a greater synthesis of chondroitin sulphate or an increase in the mass of cartilage

KARL PASCHKIS When Dr Simpson presented the admirable comparison between the findings in growth hormone treated rats and those in acromegalics I planned to ask if she had ever seen osteoporosis in the rats I would like to put the word osteoporosis in quotes This is a change frequently though not regularly seen in acromegalic skeletons After Dr Copp presented his paper I would have directed the question to him as well Does Dr Copp think that the extreme conditions under which his rats operated had anything in common with nutritional conditions in humans? It seems unlikely to me If that is not the case it would still leave the question open

as to why the rarefaction of the skeleton is such a common finding in human acromegaly since it is not found in the growth hormone treated rats

MARGARET BESNAK (University of Ottawa) I would like to refer back to a plate of Dr Selye s I think the first one which he showed was a picture of the heart amongst other organs which was very much enlarged This picture was taken of an animal which had received DOCA but he said the same results could be obtained with STH or with LAP Now we have followed the weight increase of the heart which results from experimentally produced aortic stenosis in male rats of from 150 to 200 grams Increased cardiac weights developed in otherwise normal rats while the same degree of aortic stenosis caused no weight increase in the hearts of hypophysectomized rats In regard to the hormonal replacements in our hypophysectomized animals we have used rather large doses of LAP and some growth preparations of Armour Laboratories Each rat received from 25 to 50 mg per day for 5 days With some preparations of STH we could obtain in the hypophysectomized rats the same cardiac weight increase as occurred in the normal rats while with other preparations we found no change This may be pertinent to Dr White s first remark in the sense that we have growth hormone preparations with similar effects in the growth test but have quite different effects in our study Now with these large doses in the hypophysectomized rats I did not observe hearts which were larger than in the corresponding normals If an additional strain like aortic narrowing was put to the heart however then the same dose level of the hormone would result in very large hearts

I would like to know whether Dr Selye has observed large increases in cardiac weights either in his hypophysectomized animals receiving STH or LAP or in his normal rats i e normal in the sense that there had been no unilateral nephrectomy and that sodium chloride was not used as drinking water? If so what doses of STH were used and for how long was the hormone administered?

HANS SELYE We have not obtained any cardiac enlargement with LAP or STH beyond that which was proportionate to the increase in body weight unless the animals had been specially sensitized by the high sodium chloride diet by unilateral nephrectomy or preferably by both I fully agree that some stress situation must exist as in your experiments where the hypertrophy was due to increased work against aortic stenosis Some conditioning factor must be present and in our experiments the unilateral nephrectomy was responsible

MARGARET BESNAK With the same dose level?

HANS SELYE Yes with the same level of hormone

Part III

Growth Hormone and Energy Sources

Chairman Papers 11 to 13

Francis D W Lukens

The George S Cox Medical Research Institute
University of Pennsylvania
Philadelphia Pennsylvania

Chairman Papers 14 and 15

Milton O Lee

The American Physiological Society
Washington D C

Chairman Papers 16 to 20

Charles H Best

Banting and Best Department of Medical Research
University of Toronto
Toronto Canada

11

Importance of the Nutritional State for the Biological Function of Growth Hormone

E W McHenry
Department of Public Health Nutrition University of Toronto

The nutritional state of an individual is the result of the ratio of absorbed nutrients to the requirements of the individual The amount of a nutrient absorbed depends upon the quantity ingested and upon the efficiency of digestion and of absorption The requirements of a given individual can be varied by metabolic changes induced by hormones by activity, or indeed by the amount of a nutrient which has been absorbed To cite one example of the last factor requirement for vitamin B_6 varies directly with absorbed protein Since only approximate methods are available for the estimation of nutritional state descriptions of it in experimental work on animals are limited generally to observations on changes in appearance in body weight or in some particular metabolite Descriptions of nutritional state of human subjects are frequently voluble but meaningless This discussion will be restricted to the effects of ingested food on the biological activity of growth hormone

Calorie Intake

It is assumed generally that the First Law of Thermodynamics is valid for biological systems For this reason the weight gain produced by growth hormone must result from either an increased intake or a decreased catabolism or more efficient energy production An effect of calorie intake on response to growth hormone might be expected However it is clear that growth hormone can produce effects without alterations in calorie intake and indeed in the absence of food ingestion As early as 1934 Lee and Schaffer[1] recognized that the effect of growth hormone on body weight might be due to increased consumption of food and they used isocaloric feeding Rats treated with growth hormone showed a better appetite than

greater efficiency of food utilization and that the extra weight gain offered a good method for testing the nutritive value of a diet

In a separate study Deuel and associates[13] found that growth hormone caused no weight increase in fat deficient animals and that the hormone did not aggravate the deficiency of essential fatty acids The only observation made regarding an effect of growth hormone was in relation to body weight which did not change It is possible that biochemical studies would have shown increased nitrogen retention even in the fat deficient animals Recovery of the animals was expedited by giving growth hormone in conjunction with linoleic acid

Vitamins

In 1937 Margitay Becht and Wallner[14] gave a crude pituitary extract to rats in which body weight had been plateaued by a lack of vitamin A the extract did not cause an increase in weight Eight years later a similar lack of response in vitamin A deficient rats was reported by Ershoff and Deuel [15] However the growth hormone injections caused a marked precipitation of vitamin A deficiency symptoms and increased mortality Ershoff and Deuel did not report data on food consumption and it is reasonable to assume that the deficient rats had a definitely subnormal intake

An interesting approach to the interrelation of growth hormone to vitamins was made by Beher and Gaebler[16] who studied blood vitamin levels several tissue enzymes and excretion products in adult bitches Administration of growth hormone caused a marked decrease in the urinary output of N[1] methyl nicotinamide but there was no increase in either coenzyme I or II in red blood cells Parallel studies with testosterone showed decreased outputs of riboflavin and of ascorbic acid It was suggested that induced gains in body weight increased vitamin requirements

Lotspeich[17] gave growth hormone to two groups of rats one fed a low fat diet and the other a high fat diet and the body weight had been plateaued by deprivation of pantothenic acid In both groups the hormone caused definite gains in weight but appeared to aggravate the external signs of pantothenic acid deficiency Since there was not used a non hormone treated group similarly lacking the vitamin it is difficult to decide whether there was an actual acceleration of vitamin deficiency

In our laboratory several studies[18,19] on the possible interrelation of growth hormone and B vitamins have been carried out Initially we were particularly interested in the effects of vitamin B_6-deprivation because of the generally assumed relation of this vitamin to amino acid metabolism Our studies on vitamin B_6 have shown that dietary lack of this vitamin causes rats to unduly utilize body stores of fat and to lessen the retention of amino acids The ability to maintain body protein and indeed to synthesize new protein is not impaired As in all experimentally induced nutritional deficiencies there is a loss of appetite which markedly reduces

did controls in that the supply of food, equal to that used by the controls was consumed more quickly Despite the food restriction, hormone treated rats gained more weight than did the pair fed controls Similar results were obtained by Marx et al [2] using hypophysectomized rats with pair feeding Restriction of food intake to the non hormone, post operative level made possible a response to growth hormone in a study reported by Bennett and associates [3] In 1940 Harrison and Long[4] found that growth hormone caused a significant decrease in protein loss in fasted rats but did not diminish th loss in body fat Somewhat analagous effects have been observed in several other laboratories [6 7] A definite effect of growth hormone in rats recovering from inanition was seen by Quimby, Bartlett and Artress [8]

While it is clear that biochemical effects of growth hormone can be demonstrated in fasting rats and in those with restricted food intake the magnitude of response is related to the calorie intake Conversely, a decrease in food intake means that a smaller quantity will be available for retention and for increase in body weight This relation of calorie intake to growth hormone response will be referred to in connection with effects of nutrient deficiencies

Dietary Protein

Gordon et al[9] maintained plateaued female rats on a 6 per cent casein diet growth hormone injections caused nitrogen retention but no definite gain in weight If this diet was supplemented with methionine a similar dose *of growth hormone caused gain in weight* The same authors[10] tested the response of growth hormone in respect to 5 proportions of protein and found optimal effects on nitrogen retention and on body weight when the diet contained 24 per cent casein It should be noted that the test animals were plateaued female rats and that isocaloric feeding was employed It is interesting that the optimal ratio of dietary protein on the basis of growth hormone response is roughly equal to the ratio which has been assumed for years to be optimal for the growth of young normal rats

The quality of dietary protein was studied by Chow and Greep[11] who fed a protein free diet and 4 diets each containing different proteins After administration of growth hormone differences in body weight were observed and these were attributed to the various proteins Data on food consumption were recorded and these showed that there was a rough agreement between gain in body weight and dietary intake Consequently it is not possible to decide on this evidence whether variation in the kind of amino acid intake alters the response to growth hormone

Dietary Fat

The response to growth hormone with diets containing various types of fat was studied by Deuel et al [1] and no differences were noted In discussing the results the authors pointed out that growth hormone produced

by more efficient energy production to meet current needs or by decreased energy expenditure Hypophysectomy drastically reduces food consumption and the administration of growth hormone improves appetite and increases food consumption The resultant gain in body weight is thus augmented Even in intact rats growth hormone has at least a mild effect on appetite However the effect is proportionately small and its nullification by isocaloric feeding is not sufficiently drastic as Lee and Schaffer[1] demonstrated to prevent growth hormone from slightly increasing body weight Under such circumstances the weight increase is clear proof that growth hormone produces biochemical alterations in the animal which either decrease energy expenditure or which increase the efficiency of energy production Nevertheless an increase in body weight can be prevented by a serious restriction in calorie intake despite the fundamental metabolic effect of growth hormone The magnitude of response measured in weight gain is related to the calorie intake If weight change is the only criterion used to determine growth hormone response observations should be interpreted in the light of measurements of food consumption The conclusions of Chow and Greep[11] on the effects of several kinds of dietary protein are thus open to question

A deficiency of almost any nutrient causes a decrease in appetite and in food consumption Frequently the restriction in food use is so marked that the animal is suffering not only a deficiency of the omitted nutrient but also a marked amount of inanition Such an animal could hardly be expected to show an increase in body weight after growth hormone administration If the control is fed *ad libitum* it will show a weight response The conclusion could then be drawn that the deficiency of the omitted nutrient has prevented the biological action of growth hormone If the appetite effect of the nutrient deficiency has been marked growth hormone will not cause a weight increase in a pair fed control because of the drastic reduction in calorie intake of the control to the level of the deficient animal This was found by us in studies on vitamin B_6

Even under conditions of severely restricted food intake or indeed in fasted animals biological activity of growth hormone can be demonstrated if biochemical observations are made on the tissues of the treated animal If biochemical alterations are used as criteria of growth hormone activity our conclusions regarding the relation of nutrition to such activity may be quite different If we re examine the existing evidence on this basis we find that growth hormone can function if the food intake is drastically restricted in calories or deficient in protein in thiamine or in vitamin B_6 It is doubtful whether growth hormone can produce metabolic changes in rats deficient in pantothenic acid It is clear however that growth hormone can be shown to function not only in fasting animals but in those acutely deficient in one of several nutrients provided that biochemical criteria are used The general conclusions of Ershoff are thus, hardly valid

food intake Injections of growth hormone did not cause a weight increase in the deprived rats and possible interpretation of this failure will be discussed presently Growth hormone did not alter the proportions of proteins, fat and water in the tissues of either deprived or control animals The acrodynia which is characteristic for vitamin B_6 deprived rats was accentuated by growth hormone A typical biochemical finding in vitamin B_6 deficient rats is an elevated fasting blood urea This was further increased by growth hormone It is interesting that growth hormone caused a reduction in the quantity of vitamin B_6 in the liver

The effects of growth hormone have been studied in deficiencies of three other B vitamins (thiamine riboflavin and pantothenic acid) In rats deprived of either riboflavin or pantothenic acid growth hormone did not cause an increase in body weight but did do so in thiamine deficient animals An increase in nitrogen retention was produced by growth hormone in rats deprived of riboflavin and in the thiamine deficient animals but not in those lacking pantothenic acid In all of these three deficient states growth hormone brought about a reduction in fasting blood urea an effect opposite to that seen in vitamin B_6 deficiency

Discussion

In a fairly recent review on the interrelation of nutrition and anterior pituitary function Ershoff [0] made the following statement Considerable data are available indicating that the functions of the anterior pituitary and the target organs of its secretions are largely dependent on the nutritional state and the composition of the diet fed Somatotropin will not promote a weight increment in animals that are nutritionally deficient It is interesting to consider these statements in the light of the evidence which has been summarized in the present paper

There are reports from at least five laboratories that growth hormone will cause nitrogen retention in fasting rats and in those for which food intake has been somewhat curtailed It could be concluded that growth hormone can function even in the absence of dietary calories Surely fasting rats or even those with restricted food intake could be described as being nutritionally deficient and on the basis of Ershoff s conclusion should not respond to growth hormone

One of the reasons for conflict of opinion regarding the relation of nutrition to growth hormone response is the choice of criteria used to determine the response In a number of published reports the criterion has been an increase in body weight It has been pointed out that body weight can increase whether we call it body weight increase or growth only if there is a surplus of food after the current energy expenditure of the animal has been paid That is the meaning of the First Law of Thermodynamics when applied to animal bodies The surplus to be used for body weight increase can be obtained by an increased consumption of food or

18 Beare J Beaton J R Smith F and E W McHenry *Endocrinology* 52 396 (1953)
19 Beare J L Beaton J R and E W McHenry *Endocrinology* 55 40 (1954)
20 Ershoff B H *Vitamins and Hormones* Vol X New York Academic Press Inc 1952 79
21 Patterson J M McHenry E W and W A Crandall *Biochem J* 36 792 (1942)

One other aspect of the effect of nutritional deficiencies can be further considered It has been reported that growth hormone aggravates deficiency of vitamin A[1] of pantothenic acid[17] and of vitamin B_6[18] Because of familiarity with the findings I shall consider the last named deficiency Dietary deprivation of vitamin B_6 causes a reduction in food intake and catabolism of depot stores of fat A somewhat greater proportion of ingested amino acids are catabolized than in the control animal It has been shown in a number of laboratories that growth hormone causes the animal to burn an increased amount of fat and it has been suggested that this increased burning of fat makes possible the improved retention of protein At any rate this effect of growth hormone on fat catabolism whether it be primary or secondary is in the same direction as the effect of vitamin B_6 deficiency This may be the explanation of the aggravation of vitamin B deficiency and could apply also to a deficiency of any of the B vitamins all of which similarly affect fat utilization Vitamin A deficiency also induces a decrease in the amount of body fat[1] and the same explanation might here again be valid An alternative explanation is that growth hormone increases the load on enzyme systems for which the various vitamins are needed as co enzymes Evidence is not available to make possible a correct choice of explanation

References

1 Lee M O and N K Schaffer *J Nutrition* **7** 337 (1934)
2 Marx W Simpson M E Reinhardt W O and H M Evans *Am J Physiol* **135** 614 (1941–2)
3 Bennett L L Li C H and B Laundrie *Proc Soc Exp Biol Med* **68** 94 (1948)
4 Harrison H C and C N H Long *Endocrinology* **26** 971 (1940)
5 Whitney J E Bennett L L Li C H and H M Evans *Proc Soc Exp Biol Med* **69** 118 (1948)
6 Szego C M and A White *Endocrinology* **44** 150 (1949)
7 Milman A E and J A Russell *Endocrinology* **47** 114 (1950)
8 Quimby F H Bartlett R G and J L Artress *Am J Physiol* **166** 566 (1951)
9 Gordon G S Bennett I L Li C H and H M Evans *Proc Soc Exp Biol Med* **65** 317 (1947)
10 Gordon G S Bennett L L Li C H and H M Evans *Endocrinology* **42** 153 (1948)
11 Chow B F and R O Greep *Proc Soc Exp Biol Med* **69** 191 (1948)
12 Deuel H J Jr Hendrick C and M E Crockett *J Nutrition* **31** 737 (1946)
13 Deuel J H Jr Greenberg S M Calbert C E Savage E E and T Fukui *J Nutrition* **40** 351 (1950)
14 Margitay Becht E and E Wallner *Zeitschrift Vitaminforsch* **6** 119 (1937)
15 Ershoff B H and H J Deuel Jr *Endocrinology* **36** 280 (1945)
16 Beher W T and O H Gaebler *J Nutrition* **41** 447 (1950)
17 Lotspeich W D *Proc Soc Exp Biol Med* **73** 85 (1950)

18 Beare J Beaton J R Smith F and E W McHenry *Endocrinology* 52 396 (1953)
19 Beare J L Beaton J R and E W McHenry *Endocrinology* 55 40 (1954)
20 Ershoff B H *Vitamins and Hormones* Vol X New York Academic Press Inc 1952 79
21 Patterson J M McHenry E W and W A Crandall *Biochem J* 36 792 (1942)

12

Growth Hormone and Nitrogen Retention

Paul D Bartlett
Edsel B Ford Institute for Medical Research Henry Ford Hospital Detroit

The mechanism by which a rapidly growing immature organism retains nitrogen and accumulates body protein has intrigued investigators in the field of protein metabolism for many years and still remains one of the most challenging problems in biochemical research My own interest in nitrogen retention and growth dates back to about 1938 when as a graduate student under the stimulating direction of O H Gaebler, I had the opportunity of studying the effects of anterior pituitary preparations and iodine on nitrogen excretion creatinuria, and basal metabolism in the adult female dog[1] As I look back on this experience and on the observations which we made there stands out most vividly in my mind the profound effect of growth hormone on the overall nitrogen metabolism Here indeed was an experimental procedure which seemed made to order for the study of the mechanism of nitrogen storage and growth processes With growth hormone one could turn on so to speak, a period of nitrogen storage in the adult animal and could bring about nitrogen retention and resumption of growth processes in the immature, hypophysectomized rat As is so often the case however the suggestion and prosecution of ideas are widely separated and not until several years later did I have the opportunity to return to Dr Gaebler s laboratory in the Edsel B Ford Institute for Medical Research and resume work on the mechanism of nitrogen storage induced with growth hormone

The subject Growth Hormone and Nitrogen Retention assigned to me by the symposium committee is one of considerable magnitude and one which has been extensively reviewed by Li and Evans[2] by Russell[3] and more recently by Gaebler[4] Because of this and the fact that several aspects of the nitrogen retention problem are to be considered by other participants on this symposium I have taken the liberty of confining my

subject matter to studies from our own laboratories on the kinetics of the process I refer specifically to the characterization of nitrogen storage induced in the adult dog with growth hormone in terms of rates of protein synthesis, amino acid catabolism and protein degradation

I should like to first briefly review the protocol and results of some of our earlier studies[5] in which the kinetics of amino acid and protein metabolism were evaluated In general two types of experiments were con ducted on each of three adult female dogs In one experiment the animal was maintained in nitrogen balance on a constant dietary intake and in the other experiment nitrogen retention was induced in this animal by the subcutaneous administration of small daily doses of anterior pituitary growth hormone At the desired time during the experiment in which the animal was in nitrogen balance and on the fourth day of the nitrogen storage experiment the diet was supplemented with 32 5 atom per cent excess N^{15} labeled glycine in doses of 10 mg per kg of body weight Col lections of urine were then made at 3 6 9 12 24 and 48 hours after administration of the labeled amino acid Total urinary nitrogen excretion and the N^{15} enrichment of the total urinary nitrogen were determined in each collection and from such data rates of protein synthesis and size of the nitrogen pool were evaluated by the mathematical treatment of Sprinson and Rittenberg [6] and rates of amino acid catabolism by the mathematical treatment of Hoberman [7]

Table 1

RATE OF PROTEIN SYNTHESIS AMINO ACID CATABOLISM AND SIZE OF THE NITROGEN POOL

Dog 44

	N Balance	*N Storage**
Weight Gain (kg)	None	0 23
Nitrogen Stored (g)	−0 28	10 06
Rate of Protein Synthesis (g N/kg Body Wt /24 hr)	0 49	0 86
Fraction of Amino Acid N Catabolized and Excreted (%/hr)	3 85	1 26
Size of Nitrogen Pool (g N/kg Body Weight)	0 89	1 30

* N storage induced with 5 mg growth hormone per day for five days

Results of studies on Dog 44 are summarized in Table 1 During the experiment in which nitrogen storage was induced with growth hormone both the rate of protein synthesis and the size of the nitrogen pool were

increased while the rate of amino acid catabolism expressed in terms of the percentage of amino acid nitrogen catabolized and excreted per hour was markedly reduced These results were confirmed in Dogs 52 and 55[1]

Now in the kinetic analysis of processes as complex as amino acid and protein metabolism, certain simplifying assumptions and approximations must be made to permit mathematical treatment of the postulated metabolic sequence Oversimplification might thus be expected in the first analysis and as has been recently demonstrated [8, 9] such was clearly the case in the assumption made by Sprinson and Rittenberg[6] that the rate of excretion of N^{15} in the total urinary nitrogen could be used as a measure of the rate of protein synthesis and the size of the nitrogen pool In the further analysis of this aspect of amino acid kinetics and protein metabolism in the human San Pietro and Rittenberg[9] have shown that the rate of excretion of urea is a rate determining step Values for the size of the nitrogen pool (calculated from equations which took into consideration the size of the urea pool the rate of clearance of urea from its pool and the time at

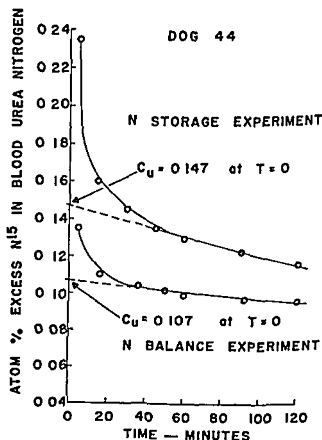


FIG 1 Effects of growth hormone on the N^{15} concentration of blood urea nitrogen following intravenous administration of labeled urea

which isotope concentration of the urea pool reached a maximum) were found to be more in accord with the total free amino acid nitrogen content of the human Rates of protein synthesis were found to be somewhat higher than previously reported[6,9] In view of these developments in the treatment of the kinetics of amino acid and protein metabolism it seemed highly desirable to again determine the size of the nitrogen pool and the rate of protein synthesis in the same series of dogs used in the earlier studies[5] In this respect the results of our most recent investigations are now to be presented We have determined the effects of growth hormone on the size of the urea pool in Dogs 44 52 and 55 and have completely evaluated once more the size of the nitrogen pool and the rate of protein synthesis in Dog 44 during typical nitrogen balance and nitrogen storage experiments

In measurements of the size of the urea pool a trace dose of N^{15} labeled urea is administered intravenously and the extent of dilution of the labeled urea in the blood determined as described by San Pietro and Rittenberg[8] In Figure 1 are presented typical data in plot form on the N^{1} enrichment of the blood urea nitrogen versus time of withdrawal of the specimen during the nitrogen balance experiment and during the experiment in which nitrogen storage was induced with growth hormone Extrapolation of the linear portion of the curve to zero time (indicated by the broken line) gives a value C_u which is taken as the N^{15} enrichment of the blood urea nitrogen corresponding to instantaneous dilution It will be noted that

Table 2

EFFECTS OF GROWTH HORMONE ON BLOOD UREA NITROGEN AND SIZE OF THE UREA POOL

Dog Number	*Blood Urea Nitrogen*	*Si e of Urea Pool*
	Nitrogen Balance	
	mg %	*g N*
44	13 80	1 28
52	9 96	0 79
55	8 80	1 10
	Nitrogen Storage	
44	10 20	0 92
52	5 86	0 50
55	6 02	0 78

* Nitrogen storage induced in all animals with 5 mg growth hormone per day for a period of 4 days and the size of the urea pool determined on the 4th day

the N^{15} enrichment of the blood urea nitrogen of this animal is higher during the nitrogen retention experiment than during the experiment in which the animal was maintained in nitrogen balance This indicates that the trace dose of N^{15} labeled urea enters and mixes with a pool of unlabeled urea which is relatively smaller during the nitrogen storage experiment These results were confirmed in two other animals used in the series From N^{15} enrichment values of blood urea nitrogen at zero time and from the number of milliequivalents of N^{15} introduced into the urea pool at zero time the size of the urea pool is calculated by the usual isotope dilution procedures In Table 2 are summarized these data for the three dogs It will be noted that the size of the urea pool is substantially reduced during nitrogen storage as is blood urea nitrogen [10]

Results of a detailed study on the rate of utilization of the nitrogen of N^{15} labeled glycine are presented in Table 3

Table 3

EFFECTS OF GROWTH HORMONE ON THE RATE OF UTILIZATION OF GLYCINE NITROGEN BY THE ADULT DOG

Time	*Urea Nitrogen Excreted*	*N^{15} Concentration Urea*	*Ammonia*	*Per Cent N^{15} Administered Excreted as Urea Nitrogen*	
		Dog 44 Nitrogen Balance			
Days	*mg*	*Atom % Excess*	*Atom % Excess*	*Per Cent*	*Cumulative Per Cent*
0 –0 022	58	0 056	3 055	0 23	0 23
0 022–0 065	179	0 153	0 577	1 92	2 15
0 065–0 091	151	0 140	0 158	1 48	3 63
0 091–0 151	516	0 107	0 060	3 88	7 51
0 151–0 193	433	0 078	0 046	2 37	9 88
0 193–0 245	579	0 067	0 037	2 72	12 60
0 245–0 309	717	0 054	0 031	2 72	15 37
0 309–0 943	2650	0 049	0 036	9 12	24 44
0 943–2 000	5425	0 018	0 016	6 85	31 29
		*Dog 44 Nitrogen Storage**			
0 –0 026	64	0 040	no data	0 18	0 18
0 026–0 044	51	0 075	0 742	0 27	0 45
0 044–0 085	176	0 094	0 352	1 16	1 61
0 085–0 157	454	0 078	0 127	2 48	4 09
0 157–0 236	589	0 065	0 055	2 70	6 79
0 236–0 330	716	0 049	0 035	2 46	9 25
0 330–0 955	2660	0 041	0 030	7 66	16 91
0 955–1 945	3535	0 018	0 018	4 47	21 38

* Nitrogen storage induced with 5 mg growth hormone per day for 5 days N^{15} labeled glycine administered on 4th day of hormonal stimulation

It can readily be seen that both the quantity of urea excreted and the per centage of administered N^{15} excreted as urea nitrogen are reduced during

nitrogen storage induced with growth hormone N^{15} enrichment of urea nitrogen reaches a maximum between 0 022 – 0 065 days in the nitrogen balance experiment, and 0 044 – 0 085 days in the nitrogen storage experiment

In connection with the amount of N^{1} in urinary ammonia two observations seem of interest In both nitrogen balance and nitrogen storage experiments the N^{15} enrichment of this fraction was found to be higher than that for urea during the first few hours following administration of the labeled glycine This is as one would expect since the size of the glutamine pool from which urinary ammonia is derived is relatively small compared with the size of the urea pool The second observation which seems of greater interest because it may indicate changes in the size of the glutamine pool and thus reflect changes in glutamine metabolism or amino acid metabolism is concerned with the relative N^{15} enrichment of urinary ammonia during the nitrogen balance and nitrogen storage experiments In the present study the N^{1} content of urinary ammonia excreted during the first few hours following administration of labeled glycine is higher during nitrogen storage than during nitrogen balance This suggests that the freshly formed glutamine labeled with N^{15} in the amide group enters and mixes with a pool of unlabeled glutamine which is relatively smaller during nitrogen storage than during nitrogen balance In confirmation of earlier studies [5] both the rate of protein synthesis and the size of the nitrogen pool evaluated as described by San Pietro and Rittenberg [9] were found to be higher during nitrogen storage induced with growth hormone than during nitrogen balance These data are summarized in Table 4

Table 4

EFFECTS OF GROWTH HORMONE ON THE RATE OF PROTEIN SYNTHESIS AND THE SIZE OF THE NITROGEN POOL

Dog 44

	N Balance	*N Storage**
Rate of Protein Synthesis (g N/kg Body Wt /24 hr)	1 65	3 00
Size of the Nitrogen Pool (mg N/kg Body Wt)	2?	89 7
Size of the Nitrogen Pool (g N/Total Dog)	0 346	1 40

* See footnote to Table 3

In an effort to obtain as complete a picture as possible of the kinetics of the alterations which growth hormone produces in nitrogen metabolism of the adult dog we have also determined rates of protein degradation and protein loss [11] The procedure which was followed and treatment of data

were essentially the same as described by Hoberman[1] In general the animals were maintained on a constant intake of stock diet and as soon as daily measurements of body weight were constant and determinations of 24 hour urinary total nitrogen indicated that the animal was in nitrogen balance a period of fasting was begun and continued for 6 days N^{15} labeled glycine was administered intravenously at the beginning of the second day of the fast and the excretion of the N^1 label was determined in the urinary nitrogen for each day during the subsequent 5 day period In the experiments concerned with measuring the effects of growth hormone on rates of protein degradation and protein loss the hormone was administered subcutaneously for a period of 5 days beginning with the second day of the fast Rates of protein degradation and protein loss were calculated for the period between the fifth and sixth days of the fast corresponding to the fourth and fifth days of hormonal stimulation In general the percentage of administered N^1 excreted in the total urinary nitrogen was reduced during the second third fourth and fifth days of hormonal stimulation This observation seems all the more striking since the decrease occurred during the period when protein stores in the fasting animal were probably being mobilized to supply carbohydrate and to meet the caloric and general metabolic requirements The average rate of protein degradation for the three dogs was found to be 6 97 ($\pm$0 17) $\times 10^{-3}$ per hour and the average rate of protein loss 3 03 ($\pm$0 70) $\times 10^{-3}$ per hour during fasting experiments Stimulation of the same animals with 5 mg of growth hormone per day for a period of 5 days reduced the average rate of protein degradation to 6 07 ($\pm$0 41) $\times 10^{-3}$ per hour and the average rate of protein loss to 2 27 ($\pm$0 57) $\times 10^{-3}$ per hour Statistical analysis of these data by the methods of Fisher[13] for small samples indicates that although the rates were reduced the differences were not significant at the P level of 0 01 Since however a P value between 0 02 and 0 05 was obtained in the statistical analysis of the rate of protein degradation data it might well be that similar data from a larger series of animals would produce a significant alteration

In view of the theoretical nature of the data on kinetics of amino acid and protein metabolism I should like to summarize briefly some results of closely related studies on the adult dog which have been obtained by more conventional procedures In respect to the rates of amino acid catabolism and the size of the nitrogen pool the effects of growth hormone on plasma glutamine and total free amino acids[14] seem of special interest In this study a sharp drop in glutamine amide nitrogen of plasma was observed in the first 24 hour period immediately following the subcutaneous administration of a 200 mg dose of growth hormone The carboxyl nitrogen of total free amino acids in plasma increased reaching a maximal value during the second 24 hour period following hormonal stimulation One may consider these findings as being in agreement with the above mentioned observations

of a reduction in the rate of amino acid catabolism during nitrogen storage induced with growth hormone The alteration in the level of free amino acids in plasma is in agreement with the increase found in the size of the metabolic pool of nitrogen measured with the isotope technique Since the latter observations were made during a period of nitrogen retention with an elevated rate of protein synthesis we are inclined to favor the view that the decrease in the rate of amino acid catabolism is a primary effect of growth hormone and not a secondary effect resulting from an increased incorporation of amino acids into proteins

In connection with some of the earlier N^{15} kinetic studies[5] we also investigated the effects of growth hormone on the concentration of plasma protein and the uptake of N^{15} in the primary fractions separated from plasma by method 10 of Cohn and associates The induction of nitrogen storage in the adult dog with growth hormone resulted in higher plasma protein concentrations in both the globulin rich F–I + II + III and the albumin rich F – IV + V fractions Evaluation of N^{15}-uptake data in terms of specific activities expressed as per cent of fed N^{15} per gram of protein nitrogen indicated that the N^{15} was incorporated into both the albumin rich and globulin rich fractions more rapidly during nitrogen storage than during nitrogen balance[15] Thus alterations in plasma proteins are in agreement with the observation of an increased rate of protein synthesis in the adult dog during induction of nitrogen storage with growth hormone

In addition to these studies I should also indicate that we have calculated the nitrogen content of total free amino acids of an animal in nitrogen balance and found this value to be in excellent agreement with the size of the nitrogen pool determined from N^{15} excretion data Thus for example assuming that the concentration of free amino acids in total body water is the same as that in blood we have calculated the nitrogen content of total free amino acids in this animal to be 0 40 g or approximately 25 mg per kg of body weight This was derived from an experimentally determined level of free amino acid nitrogen in plasma which averaged 4 4 mg per cent and from total body water of 57 8% of the body weight This agrees excellently with a value of 22 mg of nitrogen per kg of body weight obtained by the kinetic treatment of N^{15} excretion data Studies now in progress in our laboratories will soon permit a comparison of similar data obtained during nitrogen storage induced by growth hormone

Briefly summarized results of kinetic studies and more conventional procedures appear to indicate that the growth hormone induced nitrogen storage in the normal adult dog maintained on a constant dietary intake is characterized by a higher rate of protein synthesis a lower rate of amino acid catabolism a smaller urea pool and a larger metabolic pool of nitrogen than is observed in the same animal maintained in nitrogen balance Stimulation of the fasting adult dog with growth hormone reduced the rate of protein degradation

References

1 Gaebler O H and P Bartlett *J Biol Chem* **129** 559 (1939)
2 Li C H and H M Evans *Recent Progress in Hormone Research* New York Academic Press Inc **III** 3 (1948)
3 Russell J A *Protein Metabolism Hormones and Growth* New Brunswick N J Rutgers Univ Press 1953 46
4 Gaebler O H *Symposium on Protein Metabolism* New York The National Vitamin Foundation Inc 1954 38
5 Bartlett P D and O H Gaebler *J Biol Chem* **196** 1 (1952)
6 Sprinson D B and D Rittenberg *J Biol Chem* **180** 715 (1949)
7 Hoberman H D *Yale J Biol and Med* **22** 341 (1950)
8 San Pietro A and D Rittenberg *J Biol Chem* **201** 445 (1953)
9 San Pietro A and D Rittenberg *J Biol Chem* **201** 457 (1953)
10 Bartlett P D Madoff M and O H Gaebler *Division of Biological Chemistry of the American Chemical Society Abstracts of Papers Presented at New York* N Y 57C Sept 12–17 1954
11 Bartlett P D and A Stevenson *Endocrinology* **55** 200 (1954)
12 Hoberman H D *J Biol Chem* **188** 797 (1951)
13 Fisher R A *Statistical Methods for Research Workers* 10th ed London Oliver & Boyd Ltd 1948
14 Bartlett P D Gaebler O H and A Harmon *J Biol Chem* **180** 1071 (1949)
15 Bartlett P D and O H Gaebler *J Biol Chem* **196** 11 (1952)

13

Effects of Growth Hormone on the Metabolism of Amino Acids*

Jane A Russell
Division of Basic Sciences in the Health Services Emory University Georgia

Because one of the principal end effects of the action of growth hormone is the synthesis of new protein the nature of the role of growth hormone in the metabolism of amino acids occupies a central position in any discussion of the mechanism of action of this hormone A primary question is does growth hormone by some means favor the synthesis of protein from amino acids does it inhibit the breakdown of protein to the amino acid stage or does it indirectly enable the building up of more protein by slowing the catabolic destruction of amino acids? Most of the available evidence tends to support the first of these possibilities that of enhancing the uptake of amino acids into the tissues in the form of protein although perhaps it is still not possible to make a final decision as to the biochemical site of action of the hormone

Against the probability that growth hormone inhibits the catabolism of amino acids several arguments may be raised For one thing the immediate effect of the administration of growth hormone is to depress the level of amino nitrogen in plasma and liver[1 2] and possibly in other tissues In illustration a selection from observations made on the non protein nitrogen components of the liver 4 hours after the injection of a single dose of growth hormone is shown in Table I Here it may be seen that both free amino nitrogen and the sum of amide and ammonia nitrogen were significantly diminished in concentration after growth hormone The effect on circulating amino nitrogen may be seen also in nephrectomized in adrenalectomized or in alloxan diabetic animals [2 3 4] it appears probably to be a fairly direct result of growth hormone action If protein hydrolysate is given to growth

* Supported by a grant in aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council

hormone treated animals the rate of removal of exogenous amino acids from the blood is not diminished If anything it is increased[3] and the effect on amino and amide nitrogen in liver is just as evident when casein hydrolysate is given as when it is not (Table 1) If the catabolism of amino acids were inhibited at any stage by the hormone, one would expect to see an increase in the concentration of one or more of these materials in blood or liver and not the diminution which has been observed

Table 1

EFFECT OF GROWTH HORMONE ON LIVER NON PROTEIN NITROGEN IN FASTING RATS*

	Untreated†	Growth Hormone† 1 mg /100 g 4 Hours before Sampling	Difference
Fasting Rats		*mg per cent Nitrogen*	
Amino N	55	47	−8 ± 1 7‡
Amide + Ammonia N	18	13	−5 ± 0 7
Rats fasted then given casein hydrolysate (10 mg N per 100 g i v) 1 5 hrs before sampling			
Amino N	54	48	−6 ± 1 8
Amide + Ammonia N	22	16	−6 ± 2 6

* Ostashever and Russell unpublished observations
† 7–10 observations in each group
‡ Standard error of difference

A second point is that an effect of growth hormone on disposition of amino acids may be demonstrated in eviscerated functionally hepatectomized animals In the rat so prepared and maintained with glucose pituitary extract or growth hormone alone usually cannot be shown to affect the rate of release of amino acids from peripheral tissues[5] (Fig 1) As shown also in Figure 1 when an amino acid mixture is given intravenously to such a preparation the concentration of amino nitrogen in the blood plasma at first is very high as would be expected Then as the amino acids are distributed through the tissues the concentration in the blood falls to a plateau 1 to 2 hours after the infusion and before the continuing release of amino nitrogen from the tissues again becomes evident If animals given amino acids in this fashion have been pretreated with growth hormone a short time before operation the level to which the blood or plasma amino nitrogen falls at the intermediate point is lower than in the controls and it remains lower for the next few hours The differences engendered by growth hormone treatment in such circumstances although not very large are quite consistent[5] As a result of the growth hormone

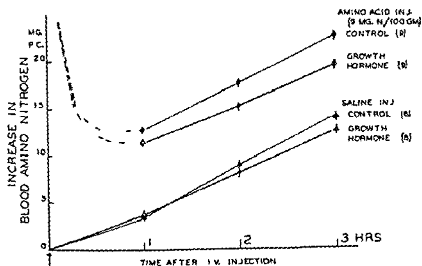


FIG 1 Effect of growth hormone on removal of amino nitrogen from the blood in eviscerate functionally hepatectomized nephrectomized rats (from Russell) [5]

influence administered amino acids are removed from the circulation in the absence of liver function Since the liver is the major site of deamination of amino acids it does not seem likely that a principal mechanism of action of growth hormone could be the inhibition of amino acid catabolism or of urea formation

That growth hormone inhibits proteolysis does not seem probable either although the evidence here is rather indirect For one thing it is quite difficult to demonstrate effects of growth hormone on the rate of nitrogen catabolism in the fasting animal although it can sometimes be done with energetic treatment during a day or more In short term experiments neither the release of amino acids from tissues in eviscerate preparations nor the rate of urea formation in nephrectomized animals is substantially affected Under otherwise identical conditions but in presence of exogenous amino acids however growth hormone brings about an increased rate of removal of amino acids from the blood and at the same time diminishes the rate of their catabolism to urea (Fig 2) In acute experiments as in long term observations the effectiveness of growth hormone in intact animals thus appears contingent on the supply of amino acids

Further indication of the relationship of the continuing supply of amino

hormone treated animals the rate of removal of exogenous amino acids from the blood is not diminished If anything it is increased[3] and the effect on amino and amide nitrogen in liver is just as evident when casein hydrolysate is given as when it is not (Table 1) If the catabolism of amino acids were inhibited at any stage by the hormone one would expect to see an increase in the concentration of one or more of these materials in blood or liver and not the diminution which has been observed

Table 1

EFFECT OF GROWTH HORMONE ON LIVER NON PROTEIN NITROGEN IN FASTING RATS

	Untreated†	*Growth Hormone† 1 mg /100 g 4 Hours before Sampling*	*Difference*
Fasting Rats		*mg per cent Nitrogen*	
Amino N	55	47	−8 ± 1 7‡
Amide + Ammonia N	18	13	−5 ± 0 7
Rats fasted then given casein hydrolysate (10 mg N per 100 g i v) 1 5 hrs before sampling			
Amino N	54	48	−6 ± 1 8
Amide + Ammonia N	22	16	−6 ± 1 6

* Ostashever and Russell unpublished observations
† 7–10 observations in each group
‡ Standard error of difference

A second point is that an effect of growth hormone on disposition of amino acids may be demonstrated in eviscerated functionally hepatectomized animals In the rat so prepared and maintained with glucose pituitary extract or growth hormone alone usually cannot be shown to affect the rate of release of amino acids from peripheral tissues[5] (Fig 1) As shown also in Figure 1, when an amino acid mixture is given intravenously to such a preparation the concentration of amino nitrogen in the blood plasma at first is very high as would be expected Then as the amino acids are distributed through the tissues the concentration in the blood falls to a plateau 1 to 2 hours after the infusion and before the continuing release of amino nitrogen from the tissues again becomes evident If animals given amino acids in this fashion have been pretreated with growth hormone a short time before operation the level to which the blood or plasma amino nitrogen falls at the intermediate point is lower than in the controls and it remains lower for the next few hours The differences engendered by growth hormone treatment in such circumstances although not very large are quite consistent [5] As a result of the growth hormone

The important point here is that the action of growth hormone was evident only during the active metabolism of amino acids In these circumstances it would appear that the hormone affected the metabolism of the administered amino acids rather than the catabolism of proteins already present in the body

Additional evidence that growth hormone does not affect the liberation of amino acids from body protein may be drawn from the data of Hoberman[6] In fasting animals given isotopic glycine and growth hormone over a period of days the total nitrogen excretion was reduced significantly in the presence of the hormone At the same time the excretion of isotope was diminished proportionately Thus there was no change in the concentration of excreted isotopic nitrogen and accordingly no apparent alteration in the contribution of non isotopic nitrogen from the body during this time

Another set of observations of a different type perhaps may be interpreted in a similar light Volk and Lazarus[7] reporting observations made in fasting rats could not demonstrate a significant effect of growth hormone treatment on nitrogen excretion When however the rate of nitrogen catabolism had been about doubled by the administration of phloridzin then the same and rather small amount of growth hormone was able to reduce the nitrogen excretion sharply If the increased amounts of amino acids coming from body tissues in these conditions may be considered as a contribution to the supply of nitrogeous compounds available for anabolism under the influence of growth hormone this observation becomes explicable

A final consideration relevant to this discussion pertains to the nature of the amino acids available during growth hormone action and to how it conditions this action If the effect of growth hormone were to either inhibit the catabolism of amino acids in general or the formation from them of urea or equally if it were to depress proteolysis the effectiveness of the hormone ought not to be altered much whatever assortment of amino acids is presented Yet it has been clearly shown in nitrogen balance experiments that the action of growth hormone is limited not only by the amount of nitrogen given but greatly by the availability of an essential amino acid methionine[8] Parallel evidence in respect to the acute action of growth hormone has not yet been obtained but there are indications that the effectiveness of growth hormone on the rate of urea formation observed in the nephrectomized rat also is influenced by the types of amino acids given A difference in behaviour of two types of protein hydrolysate has already been mentioned In another experiment when a single amino acid alanine was given instead of the hydrolysate little effect of growth hormone could be seen Further observations on the role of the different amino acids in the nitrogen retaining action of growth hormone will be of much interest in respect to the mechanism of action

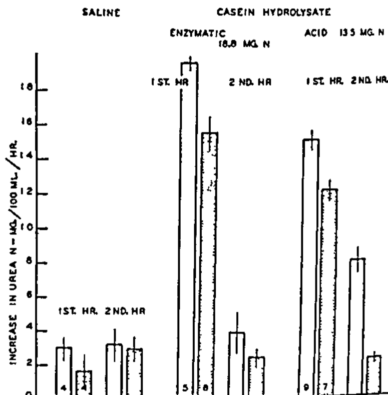


Fig 2 Effect of growth hormone on the increase in blood urea nitrogen in nephrectomized rats (3–5 hours post operative) with and without intravenous administration of protein hydrolysate and with and without growth hormone (1 mg per 100 g 1 hour before first blood sample) Figure at foot of column indicates number of observations in the group vertical line at top indicates standard error

acids to the action of growth hormone is seen in the comparison of results obtained in the nephrectomized animal with two types of casein hydrolysate also shown in Figure 2 Here it may be seen that an enzymatic digest (Amigen® Mead Johnson) and an acid hydrolysate fortified with tryptophane (Parenamine® Winthrop Stearns) differed in their effects on the rate and duration of urea production Although a larger amount of nitrogen was given in the enzymatic digest the increase in urea formation did not persist past the first hour, as it did with the acid digest The plasma amino nitrogen fell to about the same levels in the two experiments so that apparently the amino nitrogen from the enzymatic digest had been removed from the blood more rapidly and stored to a greater extent in the tissues

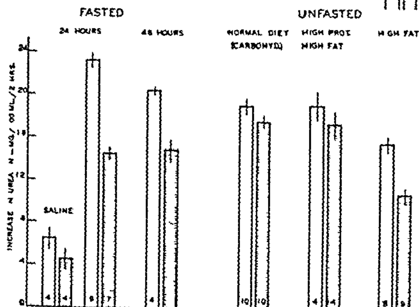


FIG 3 Effect of growth hormone on the increase in blood urea nitrogen after casein hydrolysate (except saline control at left) in nephrectomized rats Unfasted animals allowed food *ad lib* until immediately before operation had been fed either the usual diet (Purina Laboratory Chow) or for the last 4 days semi synthetic diets containing respectively 40 per cent fat 40 per cent protein and 10 per cent carbohydrate or 60 per cent fat 20 per cent protein and 10 per cent carbohydrate

the diet was low in carbohydrate but high in protein (40 per cent casein) and fat a similar result was obtained no significant effect of growth hormone was evident in the unfasted animal The rats were then fed for a few days a diet very high in fat low in carbohydrate or its precursors and containing adequate protein (20 per cent casein) In this case the rate of urea formation in the control animals was lower than in any other series but when growth hormone was given further retention of nitrogen was clearly evident These results suggest that not only is ingested carbohydrate not required with the amino acids if growth hormone is to be effective in an acute experiment but that the action of the hormone is more easily demonstrated if fat rather than carbohydrate or its precursors is furnishing the principal source of energy

Further experiments of similar type were next carried out to determine if the acute administration of carbohydrate to a fasting animal would affect the action of the hormone (Fig 4) In parallel experiments in which either a fat emulsion or glucose solution was fed by tube shortly before the opera-

If from these lines of evidence, it is considered that growth hormone does not directly affect the catabolism of either amino acids or body proteins we are left with the most likely alternative that growth hormone acts upon some phase of protein synthesis from amino acids The experiments discussed below have been interpreted in this light

Whatever the site or mechanism of action of growth hormone but perhaps if it is concerned particularly with anabolism, the synthesis of protein which results is a process requiring energy A question of some importance is whether or not there is a preferential source of energy during the nitrogen retention of growth hormone action As has long been known there is an intimate although still ill defined relationship between nitrogen balance and the metabolism of other food stuffs Carbohydrate is generally considered to be protein sparing but fat also may act in the same manner under certain conditions[9] With this in mind is either carbohydrate or fat to be considered a protein sparing agent when growth hormone is effecting nitrogen retention? In observations on the disposition of administered amino acids in nephrectomized rats receiving growth hormone evidence has recently been obtained which may provide partial answers to these questions

In all of the presently reported experiments the technique was that previously described[10] Normal adult rats were nephrectomized given a standard amount of casein hydrolysate (Parenamine ® Winthrop Stearns) and the rate of urea formation measured during the following hours A single dose of growth hormone (1 mg per 100 g body weight) was given shortly before the amino acid mixture The difference in amount of urea formed in parallel observations on control and hormone treated animals was taken as an indication of the degree of nitrogen retention induced by growth hormone under the conditions of the experiment

The initial observations of this type had all been made upon animals fasted 24 hours before the experiment with results similar to those shown in Fig 3 Of interest alone is the fact that such a clear indication of growth hormone action could be obtained in the absence of foodstuffs other than the injected amino acids Even a fast of 48 hours did not greatly alter the effectiveness of growth hormone in this respect It is evident then that exogenous carbohydrate is not required for growth hormone action on the metabolism of administered amino acids

Attention was next directed to the possible influence of foodstuffs on the action of the hormone Instead of using animals which had been fasted overnight we made our observations on animals which had been eating their normal diet until the morning of the experiment We were somewhat surprised to find that the usual effect of growth hormone on urea formation was scarcely evident This diet had been of the usual sort rather high in carbohydrate (Purina Laboratory Chow) Accordingly the experiments were next repeated on animals fed diets of different composition Where

hydrate diet thus affecting perhaps the action of growth hormone (Fig 5) In one series rather large amounts of sodium β-hydroxybutyrate were given subcutaneously a short time before and during the period of metabolic utilization of casein hydrolysate The nature of this salt and its high osmotic concentration required the administration of a large volume of material Accordingly the experiments were not entirely satisfactory from a physiological standpoint and as one type of control sodium acetate was given in similar molar mounts From the data it appears that a significant effect of growth hormone could be obtained in the unfasted animal when it was given these large amounts of a partially oxidized intermediate in fat metabolism

In a further set of observations in this series an emulsion of vegetable oil in water was injected intravenously about one half hour before the

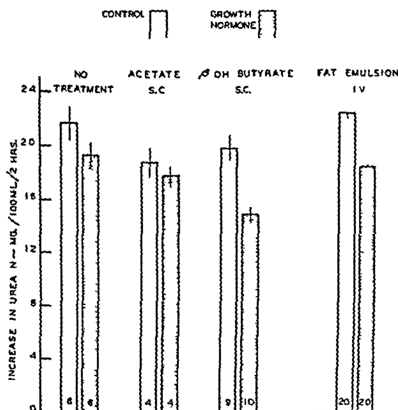


Fig 5 Effect of growth hormone on the increase in urea nitrogen after casein hydrolysate in unfasted animals (normal diet) given sodium acetate or sodium β hydroxybutyrate (0 3–0 5 mM per 100 g in 5 per cent solution) subcutaneously or an oil emulsion (10 per cent in water 0 5 ml per 100 g) intravenously 30–40 minutes before injection of amino acids

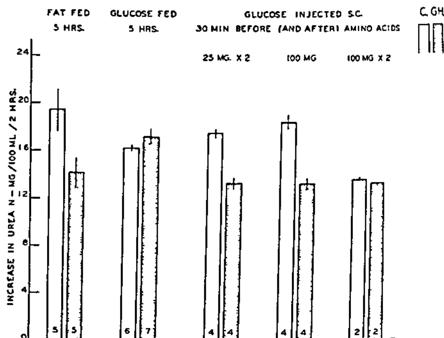


FIG 4 Effect of growth hormone on the increase in blood urea nitrogen after casein hydrolysate in nephrectomized rats fasted 24 hours then given glucose or fat Glucose (20 per cent 1 ml) or olive oil emulsion (10 per cent in water 1 ml) was fed about 1 hour preoperatively (5 hours before experiment) or glucose (5 or 10 per cent 0 5 or 1 ml) was injected subcutaneously ½ hour before experiment and in 2 of the 3 series also ½ hour after the injection of amino acids (All quantities listed were given per 100 g body weight)

tion it was found that while the prior feeding of glucose somewhat diminished the rate of urea formation from the amino acids given later it completely prevented the further reduction by growth hormone Somewhat similar results were obtained when glucose was injected more or less concomitantly with the amino acid mixture Here small amounts of glucose given before and after the hydrolysate made little difference Similar results were obtained when a larger amount was given only before the hydrolysate but in the latter instance the action of growth hormone was confined wholly to the second hour The continued administration of carbohydrate while resulting in a marked nitrogen sparing effect itself apparently prevented further activity by growth hormone Thus it appears that exogenous carbohydrate certainly does not augment the nitrogen retaining action of growth hormone and it may indeed limit the latter s effectiveness in an acute experiment

In a final series of experiments attempts were made to alter the metabolic mixture of unfasted animals which previously had been on a high carbo

tions were made and in this case the brief interval might explain the discrepancy The differences here are not large and perhaps no great weight may be attached to them If the concentration of ketone levels in the blood may be taken as a qualitative indication of the participation of fat in the metabolic mixture however these data substantiate other evidence of some degree of parallelism between the nature of the metabolic substrate and the effectiveness of growth hormone on nitrogen retention

The observations presented here invite some speculation concerning the normal role of the pituitary growth factor in nitrogen metabolism We know that one of the requirements for nitrogen retention and growth in mammalian species is a normal metabolism of carbohydrate as regulated by insulin It may be however that insulin is not the only hormone concerned in the usual control of nitrogen balance in the adult and that growth hormone also has a regular function in this respect

Two sets of observations may be recalled in this connection One is that the hypophysectomized animal not only fails to grow but in fact may lose nitrogen This was first shown in the now-classic observations of Lee and Ayres [11] in which control animals were limited to the same rather low voluntary food intake of the operated subjects The controls lost no nitrogen whereas the operated animals lost a large fraction of their body protein in a few weeks Long and Fry[1] have observed that if rats are hypophysectomized and then immediately fasted for 2 or 3 days the nitrogen excretion in this post operative period is higher than that of the control fasted animals We have confirmed this in a few animals On the other hand as Levin[13] and others have shown if the hypophysectomized rat is force fed ample quantities of a normal diet the loss of nitrogen which otherwise occurs may be reduced to minor proportions These observations indicate that the pituitary is essential for normal nitrogen retention at least during fasting or in periods of insufficient food if not when the food intake is high

As has been known for many years the carbohydrate and protein moieties of the diet must be fed essentially together if nitrogen balance is to be maintained in the adult although it is not certain that this is necessary for growth in the young animal Cuthbertson Webster and Young[14] some years ago made the important observation that when the intakes of dietary protein and carbohydrate were separated in time the expected loss of nitrogen could be overcome completely by the injection of a crude anterior pituitary extract rich in growth hormone These observations need now to be repeated with purified growth hormone but there can be little doubt that this hormone was the operative principle in this circumstance The results of these several experiments together with our observations presented above suggest that one of the functions of growth hormone in the normal animal might be to enable the retention of nitrogen especially during conditions when for one reason or another carbohydrate was not sufficiently available In this sense the action of growth hormone would be complementary to that of

casein hydrolysate The emulsion was prepared with some difficulty from small amounts of monostearate and bile salts Because of the nature of the emulsion and because such large volumes of material were given intravenously to nephrectomized animals—certainly not a happy necessity—the results were rather variable Even so, a significant effect of growth hormone was demonstrable if comparison was made between the control and treated animals which had been studied simultaneously These results, although not conclusive in themselves, suggest that the nitrogen anabolic effect of growth hormone can be favored by an acute shift in the metabolic mixture toward fat or its metabolites as well as by the more chronic administration of a high fat diet over a period of days

Table 2

ENERGY SOURCES AND GROWTH HORMONE ACTION
in Nephrectomized Rats Given Casein Hydrolysate

Experimental Condition		*G H Effect on Urea N*	*Blood Ketones* Av initial mg per cent*
Fasted	24 hrs	+	17
	48 hrs	+	36
Unfasted	Carbohydrate	−	7
	Protein Fat Diet	−	9
	Fat Diet	+	15
Unfasted (CHO Diet)	Acetate s c	−	8
	β OH Butyrate s c	+	18
	Fat Emulsion i v	+ ?	7
Fasted	Fat Fed 5 hrs	+	17
	Glucose Fed 5 hrs	−	13
	Glucose Inj ½ hr		
	50 mg	+	19
	100 mg	−	10

* As acetone

A final table summarizes these experiments on the sources of energy and growth hormone action (Table 2) Here it may be seen that the disposition of administered amino acids was affected by growth hormone only when fat was being used Accompanying this summary are presented the average blood ketone levels in the several experiments at the time of injection of the amino acid mixture The growth hormone treated animals exhibited somewhat higher ketone levels in all series but the data from both control and treated animals have been combined here for illustrative purposes In all but one case the ketone level was higher in conditions in which growth hormone action was evident The exception was the experiment in which fat emulsion was given intravenously only a short time before the observa

tions were made and in this case the brief interval might explain the discrepancy The differences here are not large and perhaps no great weight may be attached to them If the concentration of ketone levels in the blood may be taken as a qualitative indication of the participation of fat in the metabolic mixture however these data substantiate other evidence of some degree of parallelism between the nature of the metabolic substrate and the effectiveness of growth hormone on nitrogen retention

The observations presented here invite some speculation concerning the normal role of the pituitary growth factor in nitrogen metabolism We know that one of the requirements for nitrogen retention and growth in mammalian species is a normal metabolism of carbohydrate as regulated by insulin It may be however that insulin is not the only hormone concerned in the usual control of nitrogen balance in the adult and that growth hormone also has a regular function in this respect

Two sets of observations may be recalled in this connection One is that the hypophysectomized animal not only fails to grow but in fact may lose nitrogen This was first shown in the now-classic observations of Lee and Ayres[12] in which control animals were limited to the same rather low voluntary food intake of the operated subjects The controls lost no nitrogen whereas the operated animals lost a large fraction of their body protein in a few weeks Long and Fry[3] have observed that if rats are hypophysectomized and then immediately fasted for 2 or 3 days the nitrogen excretion in this post-operative period is higher than that of the control fasted animals We have confirmed this in a few animals On the other hand as Levin[13] and others have shown if the hypophysectomized rat is force fed ample quantities of a normal diet the loss of nitrogen which otherwise occurs may be reduced to minor proportions These observations indicate that the pituitary is essential for normal nitrogen retention at least during fasting or in periods of insufficient food if not when the food intake is high

As has been known for many years the carbohydrate and protein moieties of the diet must be fed essentially together if nitrogen balance is to be maintained in the adult although it is not certain that this is necessary for growth in the young animal Cuthbertson Webster and Young[14] some years ago made the important observation that when the intakes of dietary protein and carbohydrate were separated in time the expected loss of nitrogen could be overcome completely by the injection of a crude anterior pituitary extract rich in growth hormone These observations need now to be repeated with purified growth hormone but there can be little doubt that this hormone was the operative principle in this circumstance The results of these several experiments together with our observations presented above suggest that one of the functions of growth hormone in the normal animal might be to enable the retention of nitrogen especially during conditions when for one reason or another carbohydrate was not sufficiently available In this sense the action of growth hormone would be complementary to that of

insulin in respect to nitrogen retention It would represent a most useful function for the hormone and one unaffected apparently by other agents This is obviously an hypothesis but it is one susceptible to additional experimental study

Summary

From a summary of available evidence, it is concluded that nitrogen retention effected by growth hormone in acute experiments is not the result of an inhibition of amino acid catabolism or of protein breakdown but a result rather of the hormone apparently favoring the uptake by the tissues of amino acids as proteins The effectiveness of the hormone was shown to be dependent to a large extent on the continuing supply of amino acids for synthesis In observations made on nephrectomized rats given protein hydrolysate nitrogen retention under the action of growth hormone was found not to require the presence of exogenous carbohydrate Instead nitrogen retention was best demonstrated when fat was serving as the principal energy source The metabolism of fat thus might be considered as the protein sparing effect which possibly supplies the energy for protein synthesis during growth hormone action From these and other observations it is suggested that one of the functions of growth hormone in the adult animal might be to enable the conservation of nitrogen during conditions when the supply of carbohydrate is limited

References

1 Li C H Geschwind I and H M Evans *J Biol Chem* **177** 91 (1949)
2 Milman A E and J A Russell *Endocrinology* **47** 114 (1950)
3 Russell J A and M Cappiello *Endocrinology* **44** 333 (1949)
4 Russell J A (unpublished)
5 Russell J A *Protein Metabolism Hormones and Growth A Symposium* New Brunswick N J Rutgers Univ Press 1953 46
6 Hoberman H D *Yale J Biol Med* **22** 341 (1950)
7 Volk B W and S S Lazarus *Proc Soc Exp Biol Med* **83** 151 (1953)
8 Gordan G S Bennett L L Li C H and H M Evans *Proc Soc Exp Biol Med* **65** 317 (1947)
9 Munro H N *Physiol Rev* **31** 449 (1951)
10 Russell J A *Endocrinology* **49** 99 (1951)
11 Lee M and G B Ayres *Endocrinology* **20** 489 (1936)
12 Long C N H and E G Fry (unpublished)
13 Levin L *Am J Physiol* **141** 143 (1944)
14 Cuthbertson D P Webster T A and F G Young *J Endocrinology* **2** 459 (1941)

14

The Role of Insulin in Nitrogen Retention*

F D W Lukens and S M McCann
George S Cox Medical Research Institute and the Department of Physiology
University of Pennsylvania Philadelphia

The importance of insulin in the retention of nitrogen and in protein balance was well demonstrated by Minkowski who showed that the nitrogen excretion during fasting was doubled by pancreatectomy The correction of this exaggerated protein catabolism by insulin some thirty years later made it clear that insulin had a part in protein metabolism Houssay[1] discovered that the anterior pituitary was responsible for the excessive protein catabolism of diabetes At the same time Evans [3] and others[4] defined the remarkable anabolic power of growth hormone Li[5 6] and Wilhelmi[7] described its purification and others recorded the nitrogen retaining action of testosterone Thus at present we are aware of multiple anabolic hormones as measured by nitrogen balance studies This naturally leads to the question of where and how each hormone acts and how they are interrelated In considering the effect of insulin on protein metabolism I shall begin by asking three questions

I To what extent *is the anabolic action of growth hormone* dependent upon insulin?

II To what extent is the anabolic or protein sparing action of insulin dependent upon growth hormone? One could ask the same question about testosterone and other anabolic hormones but these will not be considered at this time

III Is the effect of insulin on protein metabolism a primary one or is it secondary to the increased utilization of carbohydrate?

The answers to any question of this magnitude will eventually be obtained by the use of several methods There are obvious limitations to infor

* Supported by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health United States Public Health Service

mation derived solely from studies of the nitrogen balance which will not reveal the simultaneous processes of anabolism and catabolism in different tissues But since knowledge of the net nitrogen balance is likely to be a useful foundation for further studies, it has been used at this stage of the work and some of the information about the three questions asked above will be outlined on this basis

Table I

RESPONSE TO GROWTH HORMONE (GH) IN HOUSSAY CATS*

No of Cats	Average Urinary Glucose		Average Urinary Nitrogen		Nitrogen Retained ± SEM
	Before GH	During GH	Before GH	During GH	
	g per day		g per day		g per day
7	2 4	4 7	2 50	2 44	0 06 ± 0 14
5	Normal cats given 3 mg GH daily†				0 90 ± 0 07

* The hypophysectomized-depancreatized cats received *no* insulin Each cat was fed a constant diet before and during the administration of growth hormone Of the Houssay cats 5 cats received 3 mg and 2 received 10 mg daily of Armour s growth hormone

† From (9)

I *To what extent is the anabolic action of growth hormone dependent upon insulin?* This question has been examined in many ways by the use of crude and later of highly purified preparations of growth hormone Gaebler and Robinson[8] first showed that in depancreatized dogs maintained on fixed doses of insulin the action of growth hormone on nitrogen balance was present but greatly reduced When the amount of insulin was increased two to three fold the normal degree of nitrogen retention occurred Milman de Moor and Lukens[9] obtained similar results in depancreatized cats In addition they observed the effect of growth hormone in the complete absence of insulin by using hypophysectomized depancreatized (Houssay) animals In the absence of insulin growth hormone failed to cause nitrogen retention The mean nitrogen retention of seven experiments shown in Table 1 is 0 06 ± 14 g per day i e essentially zero In Figure 1 is illustrated the erratic behavior of the Houssay animal In spite of these wide variations when the results are viewed together with those from depancreatized animals which have been cited it seems probable that growth hormone is almost entirely dependent on insulin for its nitrogen sparing effect This raises the possibility that growth hormone might exert all of its effect by stimulating the production of more insulin but before discussing this possibility the second question will be examined

II *To what extent is the anabolic or protein sparing action of insulin dependent upon growth hormone?* To examine this question one would

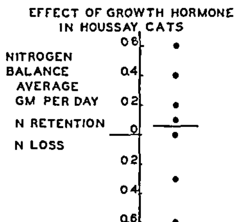


FIG 1 This shows the distribution of the experiments summarized in line 1 of Table 1

first like to know what effect insulin had on protein metabolism in the absence of growth hormone which means in the hypophysectomized animal Little work has been done on this topic but Salter and Best[10 10] have de scribed growth in hypophysectomized rats treated with insulin Bancroft Geiger and Hagerty[11] reported that the protein sparing action of carbohy drate was the same in hypophysectomized as in normal rats We have studied the protein sparing action of carbohydrate in normal and hypophy sectomized cats The purpose of these experiments was to test the effect of the insulin which is presumably secreted in response to the added glucose and yet to avoid the risk of insulin reactions in hypophysectomized animals We have begun experiments on the effect of insulin in Houssay cats

Methods

The operative and chemical methods have been described [9] The animals were kept in metabolism cages during the experimental periods and were fed measured diets of fresh horse meat which were constant for each animal throughout all periods which were compared In these experiments nitro gen retained means the difference between the amount of urinary nitrogen excreted during a control period and the amount excreted during a period of treatment The food nitrogen urinary nitrogen balance has not been used as it seemed to be less accurate than having the observed nitrogen excretion of each animal as a control Nitrogen analyses of different lots of meat have suggested that the diet was quite constant in its protein content The normal and hypophysectomized animals were fed 100 g of horse meat daily The hypophysectomized depancreatized (Houssay) animals were fed 50 or 100 g daily according to their appetites but the amount selected for each animal was constant during all periods which have been compared Like wise the addition of 2 g daily of Viokase® a desiccated pancreatic

mation derived solely from studies of the nitrogen balance which will not reveal the simultaneous processes of anabolism and catabolism in different tissues But since knowledge of the net nitrogen balance is likely to be a useful foundation for further studies, it has been used at this stage of the work and some of the information about the three questions asked above will be outlined on this basis

Table 1

RESPONSE TO GROWTH HORMONE (GH) IN HOUSSAY CATS*

No of Cats	Average Urinary Glucose		Average Urinary Nitrogen		Nitrogen Retained ± SEM
	Before GH	During GH	Before GH	During GH	
	g per day		g per day		g per day
7	2 4	4 7	2 50	2 44	0 06 ± 0 14
5	Normal cats given 3 mg GH daily†				0 90 ± 0 07

* The hypophysectomized depancreatized cats received *no* insulin Each cat was fed a constant diet before and during the administration of growth hormone Of the Houssay cats 5 cats received 3 mg and 2 received 10 mg daily of Armour's growth hormone

† From (9)

I *To what extent is the anabolic action of growth hormone dependent upon insulin?* This question has been examined in many ways by the use of crude and later of highly purified preparations of growth hormone Gaebler and Robinson[8] first showed that in depancreatized dogs maintained on fixed doses of insulin the action of growth hormone on nitrogen balance was present but greatly reduced When the amount of insulin was increased two to three fold the normal degree of nitrogen retention occurred Milman de Moor and Lukens[9] obtained similar results in depancreatized cats In addition they observed the effect of growth hormone in the complete absence of insulin by using hypophysectomized depancreatized (Houssay) animals In the absence of insulin growth hormone failed to cause nitrogen retention The mean nitrogen retention of seven experiments shown in Table 1 is 0 06 ± 14 g per day i e essentially zero In Figure 1 is illustrated the erratic behavior of the Houssay animal In spite of these wide variations when the results are viewed together with those from depancreatized animals which have been cited it seems probable that growth hormone is almost entirely dependent on insulin for its nitrogen sparing effect This raises the possibility that growth hormone might exert all of its effect by stimulating the production of more insulin but before discussing this possibility the second question will be examined

II *To what extent is the anabolic or protein sparing action of insulin dependent upon growth hormone?* To examine this question one would

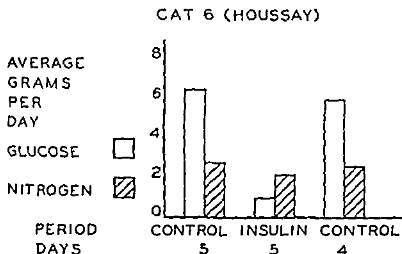


FIG 2 *Cat 6* Hypophysectomy 36 days pancreatectomy 3 days before the beginning of the first of the consecutive metabolic periods charted The diet was 50 g of meat daily throughout these periods The change in glycosuria due to insulin is obvious the average nitrogen retention of 0 45 g per day during 0 2 units of protamine insulin daily exceeds the average protein sparing action of 5 g of glucose in normal cats (cf Table 2) The three animals with less utiliza tion of glucose and no nitrogen retention were studied for similar metabolic periods

The striking contrast between insulin and growth hormone in the Houssay animal is apparent when the findings in Tables 1 and 2 are compared As noted above growth hormone appears to be ineffective in the absence of insulin yet insulin appears fully effective in the absence of growth hormone Before discussing these findings some additional facts about the experi ments with insulin in the Houssay animal may be mentioned We have found no reference to the metabolic effects of insulin in Houssay animals although the sensitivity of these animals to single doses of insulin is known Several of our animals died from hypoglycemia as we confirmed this ex treme insulin sensitivity In the experiments listed in Table 2 the dose of insulin given to Houssay cats varied but in the most recent experiments it was 0 5 unit of protamine insulin daily This may be contrasted with the doses of 10 to 20 units per day needed to maintain depancreatized cats [9] When insulin reactions were avoided the great susceptibility of Houssay cats to insulin could at times be revealed by the large amount of glucose utilized per unit of insulin As is shown in Table 3 the utilization of glucose in these experiments means the diminution in urinary glucose during the period of treatment with insulin

The data in Table 3 are based on 5 metabolic periods in the 4 animals whose nitrogen balances are summarized in Table 2 Allan[1] reported a similar range of glucose utilization by insulin in depancreatized dogs fed

preparation supplied by the Viobin Company Monticello Illinois was constant during all periods of study in the Houssay animals In the normal and in the hypophysectomized animals the metabolic periods were 6 days with rare exceptions In the Houssay animals 3 to 5 day periods were used In all experiments the effect of glucose growth hormone or insulin on nitrogen balance was based on consecutive periods However in summarizing multiple study periods in the same animal, the average results of all control and all similar treatment periods were employed Protamine insulin (Lilly) and purified growth hormone which was generously provided by The Armour Laboratories were the hormones used

Table 2

NITROGEN EXCRETION OF CATS UNDER VARIOUS CONDITIONS

No of Cats	*Average Urinary Nitrogen*		*N Retained*	*Conditions*
	Meat Only	*Meat & Glucose*		
	g /day	*g /day*	*g /day*	
16	3 38 ± 0 09	3 05 ± 0 10	0 33 ± 0 08*	Normal
13	3 25 ± 0 07	3 12 ± 0 05	0 13 ± 0 06*	Hypophysectomized
4	3 57*	*Meat & Insulin* 3 18	0 39	Houssay

* SEM

In our first report of the study of growth hormone and insulin,[9] the nitrogen sparing effect of 5 grams of glucose was easily demonstrated in normal cats That 13 hypophysectomized animals so tested displayed less than half the nitrogen retention of the normal cats is shown in Table 2 Since the difference between the nitrogen retention of the normal and hypophysectomized cats (0 20 ± 0 10 SE_{diff}) is of doubtful significance one can only say that in the insulin sensitive hypophysectomized cat this effect of glucose seems to be less than in the normal animal In this respect our cats differed from the rats studied by Bancroft et al [11] but when our results are viewed with those of Salter and Best[10 10] and of Bancroft et al [11] the data suggest that insulin can spare protein in the absence of growth hormone although this occurs to a subnormal degree in the case of the cat

In Table 2 (line 3) is summarized the effect of insulin on Houssay cats and one will note that when insulin is administered under these circumstances fully normal nitrogen retention occurs in spite of the absence of the pituitary In Figure 2 is illustrated the response of a single animal Yet when an equivalent amount of glucose is utilized by the animal s own insulin (Table 2, line 2) the nitrogen retention is deficient This emphasizes the importance of the metabolic background in studies of this sort

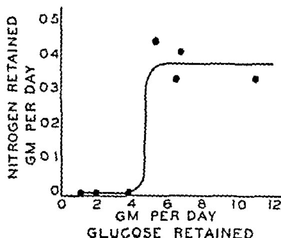


FIG 3 Relation between the increase in the amount of glucose utilized (decrease in glycosuria) and nitrogen retained during insulin treatment of hypophysectomized depancreatized (Houssay) cats The four animals which used more than 5 grams of glucose during insulin treatment are the same as those shown in Tables 2 and 3

glucose available from protein during fasting using conventional D N ratios would amount to 5 or 6 grams of glucose daily in the case of the cat This means that the nitrogen retention caused by growth hormone in fasting animals[13 14] occurs at or above the minimal limit observed in our Houssay animals

These experiments which demonstrate the essential role played by insulin in sparing protein and the apparent necessity of insulin for the action of growth hormone must be related to certain other observations Growth hormone does not act entirely by stimulating the secretion of insulin because insulin alone does not reproduce all of the effects of growth hormone There are many ways in which the nitrogen retaining effect of insulin and that of growth hormone may differ and we need only point out two areas of difference One of these is the time factor Insulin causes nitrogen retention for a week or two i e of relatively brief duration (Fig 4) In this respect it resembles the brief nitrogen retention of androgenic steroids[1] On the other hand the prolonged effectiveness of administered growth hormone has been known since the production of acromegalic dogs[3 4] as a result of months of hormone treatment Insulin and testosterone appear to set optimal levels for nitrogen equilibrium growth hormone is capable of causing a long sustained positive nitrogen balance This is also illustrated by the observation that hyperinsulinism due to islet cell adenoma of 6 to 7 years duration does not produce acromegaly

meat and 100 to 150 grams of glucose, but gave no data on dogs fed diets of meat only which are known to result in less efficient utilization of glucose. The marked diminution of glycosuria by insulin in Houssay cats is perhaps noteworthy because it occurred at unusually low insulin dosage and on regimens which ordinarily do not yield such results

Table 3

EFFECT OF INSULIN ON GLYCOSURIA OF HOUSSAY CATS

Cat No	*Diet*	*Urinary Glucose*		*Glucose Utilized by Insulin*	*Insulin* per Day*
		Meat Only	*Meat & Insulin*		
	g	*g per day*		*g /day*	*units*
6(53)	50	6 5	0 9	5 6	2 P
156	180	13 1	1 2	11 9	4 R
7a	50	7 2	0 9	6 3	0 5 P
7b	100	10 6	4 0	6 6	0 5 P
8(54)	100	10 1	2 0	8 1	0.5 P

* P = protamine insulin given once daily
R = regular insulin total of divided doses

Regardless of the dose of insulin the amount of glucose utilized per day as a result of treatment with insulin appeared to have an important relation to nitrogen retention In normal cats 5 grams of glucose added to the diet of 100 grams of meat were found empirically to have a fairly consistent protein sparing action Larger amounts of glucose (10 g per day) occasionally increased the amount of nitrogen retained but often seemed to make no difference Smaller amounts of glucose have not yet been tested in normal animals

In the case of the Houssay cats given insulin those shown in Tables 2 and 3 were selected on the basis of two criteria they survived good metabolic periods and they utilized 5 grams or more of glucose daily as did the normal cats fed glucose However we tested for metabolic periods of 4 to 5 days three other Houssay cats in which for unknown reasons the insulin given was less effective None of these cats used as much as 5 grams of glucose daily and none had significant nitrogen retention In Figure 3 the amount of glucose utilized is related to the amount of nitrogen retained in this series of 7 Houssay cats selected because the metabolic periods were satisfactory Small as this series is it suggests that in the Houssay cat under these conditions there may be a minimal limit of about 5 grams of glucose daily at or above which nitrogen is retained When less than 5 grams of glucose was retained by the insulin administered there was no nitrogen retention If there is such a minimal limit it is in accord with the concept that insulin does not act primarily on protein metabolism but acts indirectly through accelerated carbohydrate utilization One may note here that the

cate that continued study of both liver and muscle will be required to define the sites of action of insulin on protein metabolism

In summary the protein anabolic action of growth hormone and of insulin has been measured in terms of the urinary nitrogen balance Although hypophysectomy is more than simple growth hormone deficiency it has been used to observe the behavior of insulin in the absence of growth hormone Because of the great sensitivity to insulin of hypophysectomized cats the protein sparing action of carbohydrate has been studied in them and the effect of insulin has been observed in hypophysectomized depancreatized (Houssay) cats In hypophysectomized cats there was slight impairment in the nitrogen retention normally caused by the addition of dietary glucose In the Houssay cats normal nitrogen retention or better occurred when insulin reduced the glycosuria by 5 grams or more The difference in response of these two types of animal both lacking in growth hormone indicates that this response to insulin may differ under different experimental conditions and that insulin may under some conditions (e g the Houssay animal) spare protein to a marked extent in the absence of the pituitary

In contrast to this behavior of insulin the failure of growth hormone to cause nitrogen retention in the absence of insulin (Houssay animal) has been confirmed The transitory nature of the protein sparing action of carbohydrate has been demonstrated in the cat This effect which depends on the presence of insulin differs from the continued anabolism which can be produced by growth hormone Some differences in the anatomical sites of action of insulin and growth hormone have been postulated from the observations of others

Our results suggest that the presence of insulin is essential for protein anabolism and that an increased secretion of insulin is needed if the maximal response to administered growth hormone is to be obtained On the other hand insulin can cause marked nitrogen retention in the absence of growth hormone (hypophysectomy) at least under some conditions The nature of this interrelationship suggests that growth hormone may act in some way to direct the fundamental anabolic influence of insulin (glucose being available) into that duration and location of protein synthesis which is called growth

References

1 Houssay B A and A Biasotti *Endocrinology* **15** 511 (1931)
2 Evans H M and J A Long *Anat Record* **21** 62 (1921)
3 Evans H M Meyer K and M E Simpson *Memoirs Univ Cal* **11** 1 (1933)
4 Putnam T J Benedict E B and H M Teel *Arch Surg* **18** 1708 (1929)
5 Li C H Evans H M and M E Simpson *J Biol Chem* **159** 353 (1945)
6 Li C H and H M Evans *Recent Progress in Hormone Research* **3** 3 (1948)

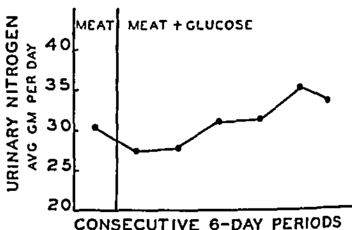


FIG 4 When 5 g of glucose were added to the constant meat intake of 100 g daily in this normal cat the nitrogen sparing action of glucose was seen only for the first two 6 day periods of added glucose

The actions of growth hormone and insulin differ not only in their duration but in their location Little is known of the sites of action of any hormones which influence protein metabolism but it is obvious that insulin has no such special effect on the bones as that produced by growth hormone It seems proper to say this about man and probably about the dog The fact that insulin is essential to the action of growth hormone has been seen in recent years by the prevention of diabetic dwarfism by insulin treatment The apparently adequate insulin supply of nondiabetic human dwarfs has clearly failed to remedy their defective growth In contrast to man, the demonstration of a positive tibia test in insulin treated hypophysectomized rats in Dr Best's laboratory[10,10] appears to be an exception due to species difference However this may be explained it is necessary to consider more than the overall or net nitrogen equilibrium as measured by urinary excretion in order to learn something of the sites of action of these anabolic hormones

The need for thinking of the nitrogen retention of particular tissues was demonstrated by Addis Poo and Lew[16] and by Munro and Naismith[17] in studies on nutrition by Greenbaum and Young[18] in connection with growth hormone and by Krahl[19] and Sinex et al [20] in terms of a specific protein synthesized *in vitro* Ulrich Tarver and Li [1] found that growth hormone exerted a striking effect on plasma proteins which in turn are formed largely or entirely by the liver The studies on liver and diaphragm *in vitro*[19,20] and the observations on the effect of glucose and insulin in hepatectomized or eviscerated animals which have been summarized by Flock, et al indi

15

Effect of Growth Hormone on Liver Proteins and Nucleic Acids

E Reid

Chester Beatty Research Institute Institute of Cancer Research Royal Cancer Hospital London S W 3

That pituitary and adrenal hormones influence the nucleic acid concentration in rat liver has been shown in a number of laboratories notably those of Li[1] DiStefano and Kosterlitz (for review see 3) Following hypophysectomy the ribonucleic acid (RNA) decreases either in absolute amount or at least relative to desoxyribonucleic acid (DNA) but administration of growth hormone (GH) prevents this change[1] The effect of adrenalectomy is similar to that of hypophysectomy

These effects on nucleic acid levels possibly reflect changes in protein synthesis a process in which the mitochondria and especially the microsomes appear to be of importance[1 5 6] (*inter alia*) Accordingly it was of interest to ascertain whether the site of these hormonal effects is the mitochondria the microsomes or the supernatant fraction In general this investigation has been concerned with hormonal effects on the yield and composition of these fractions as isolated by differential centrifugation It is indeed surprising that this technique has been extensively applied in the study of cancerous changes in liver but rather neglected in the study of endocrine influences on normal liver

---

This work was performed during tenure of a Junior Fellowship of the British Empire Cancer Campaign and was supported by grants to the Institute from the British Empire Cancer Campaign the Jane Coffin Childs Memorial Fund for Medical Research the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health U S Public Health Service I am grateful to Dr S L Steelman of The Armour Laboratories Chicago for providing growth hormone (Lot R285–183) and to Sir John Taylor of the Medical Research Council for providing cortisone acetate I express my thanks to Mr I Martin for technical assistance to Mr E Sykes for drawing the Figures and to Professor Alexander Haddow for general encouragement.

7 Wilhelmi A E Fishman J B and J A Russell *J Biol Chem* 176 735 (1948)
8 Gaebler O H and A R Robinson *Endocrinology* 30 627 (1942)
9 Milman A E De Moor P and F D W Lukens *Am J Physiol* 166 354 (1951)
10 Salter J and C H Best *Brit MJ* 2 353 (1953)
10a Lawrence R T B Salter J M and C H Best *Brit MJ* 2 437 (1954)
11 Bancroft R W Geiger E and E B Hagerty *Endocrinology* 49 149 (1951)
12 Allan F N *Am J Physiol* 67 275 (1924)
13 Harrison H C and C N H Long *Endocrinology* 26 971 (1940)
14 Bennett L L Kreiss R E Li C H and H M Evans *Am J Physiol* 152 210 (1948)
15 Stafford R O Bowman B J and K J Olson *Proc Soc Exp Biol Med* 86 322 (1954)
16 Addis T Poo L J and W Lew *J Biol Chem* 116 343 (1936)
17 Munro H N and D J Naismith *Biochem J* 54 191 (1953)
18 Greenbaum A L and F G Young *J Endocrinol* 9 127 (1953)
19 Krahl M E *J Biol Chem* 200 99 (1953)
20 Sinex F M MacMullen J and A B Hastings *J Biol Chem* 198 615 (1952)
21 Ulrich F Tarver H and C H Li *J Biol Chem* 209 117 (1954)
22 Flock E V Block M A Mann F C Grindlay J H and J L Bollman *J Biol Chem* 198 427 (1952)

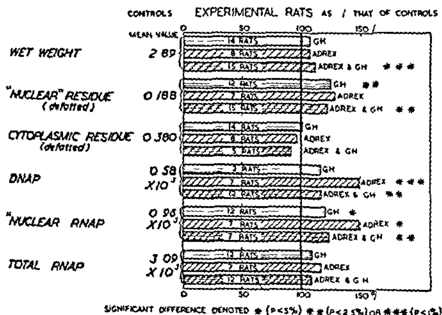


Fig 1 Data for female rats (age approx 20 weeks wt approx 250 g)

the only significant effect of GH per se was an increase in the yield of the nuclear residue and concomitantly of nuclear RNAP Possibly the homogenates from GH treated rats contained a higher proportion of un broken cells

The yields of fractions obtained by differential centrifugation are shown in Figure 2 No significant effect was obtained with GH but it is of interest that the yield of the mitochondrial fraction was decreased by adrenalectomy Recent experiments have established that this fall in the mitochondrial fraction can be reversed by administration of cortisone but cortisone also elevates total liver weight and the yield of the supernatant fraction

No such fall in the mitochondrial fraction after adrenalectomy was found in the case of kidney (Fig 3) although it is of interest that the adrenalectomized rats given GH showed a rise in relative kidney weight and a fall in the microsome fraction The data do not exclude the possibility that these effects are attributable to the adrenalectomy alone

Before discussing the composition of the liver cytoplasmic fractions corresponding data (Fig 4) will be presented for the second part of this study which comprised untreated hypophysectomized rats and also hypophysectomized rats given GH usually 0 3 mg /day for 10 days so as to reverse the growth arrest The rats were males of about 200 g weight and included not only intact or sham operated controls given the same amount of food as the

Methods

The investigation was performed with groups of albino rats kept on a diet generally fed in constant amount of which the protein content was 20% (dry weight basis) The rats in each group usually consisting of four rats including at least one control rat were all killed on the same day after an overnight fast—about three weeks postoperatively in the case of operated rats To minimize the effect of possible day to-day variations in technique the data for experimental rats have been expressed in terms of control rats within corresponding groups, rather than directly averaged over all the groups

After perfusion of the liver *in situ* with cold 0 25*M* sucrose solution under nembutal anesthesia a weighed amount of minced liver was homogenized in this medium[7] with a Potter type homogenizer The debris obtained by low speed centrifugation was homogenized and centrifuged twice more The final nuclear fraction (containing any unbroken cells) and a portion of the supernatant cytoplasmic fraction were treated with cold trichloracetic acid solution de fatted and dried for subsequent determination of nucleic acid phosphorus (DNAP and RNAP) by the Schmidt Thannhauser procedure

Centrifugation of the remainder of the cytoplasmic fraction for 20 min at 12 000 *g* (maximum value at extreme tip of tube) gave the mitochondrial fraction which was washed once Further centrifugation for 90 min at 20 000 *g* gave a microsome fraction and a supernatant fraction (cf 7) Electron microscopy kindly performed by Mr F W Cuckow, showed a clear cut difference in composition between the mitochondrial and microsome fractions Each fraction was freed from sucrose by dialysis in the cold against water this containing a trace of ammonium carbonate to prevent the pH becoming acidic [8] and was freeze dried the following day

Gross Yields, in Terms of Body Weight

In the first part of this study plateaued female rats of about 250 g weight were employed to ascertain the effects of adrenalectomy (with maintenance on salt) and of a two week treatment with GH at a daily dose of 0 6 mg (Fig 1) Not only intact rats but also adrenalectomized rats were given GH with a view to verifying that its growth promoting action was quantitatively as marked in rats lacking adrenocorticoids as in intact rats consuming the same amount of food In agreement with data in the literature no impairment of the growth response was found in the absence of the adrenals even if the ovaries were also removed but nevertheless there remains the suspicion that adrenalectomized animals may have tissue capable of secreting traces of corticoids sufficient to play a permissive role in the growth promoting action of GH

As shown in Figure 1 adrenalectomy led to a rise in liver DNAP but

experimental animals but also controls fed *ad lib* It was found that the different controls within each group could be regarded as equivalent in all respects, with the possible exception of relative liver weight In comparison with only the intact controls fed *ad lib* the hypophysectomized rats showed no increase in liver weight (cf Fig 4) although there was no indication of a decrease as noted in the laboratories of Li[1] and of Gaebler[8] The present finding that hypophysectomized rats in comparison with *pair fed* controls have an increased ratio of liver weight to body weight is in agreement with data published by Bartlett and Glynn[9] However on giving GH to hypophysectomized rats kept on a restricted food intake Bartlett and Glynn noted a decrease in liver weight contrary to the findings in the present study

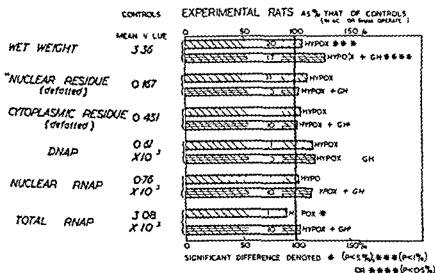


FIG 4 Data for male rats (age approx 10 weeks wt approx 200 g)

The fall in total liver RNA now observed in hypophysectomized rats given no GH (Fig 4) is in general agreement with data in the literature (e g 1) but there was no rise in DNA such as was reported from Di Stefano s laboratory

In Figure 5 are shown the yields of cytoplasmic fractions from differential centrifugation In the hypophysectomized rats given no GH as in the adrenalectomized animals there was a decrease in the mitochondrial fraction Conversely an increase in the microsome fraction was found in the hypophysectomized rats with or without GH There was no change in the yield of the supernatant fraction nor was any significant change found in the yield of an ultracentrifugal fraction as obtained from the supernatant in a few experiments by further centrifugation for 90 minutes at 145 000 *g*

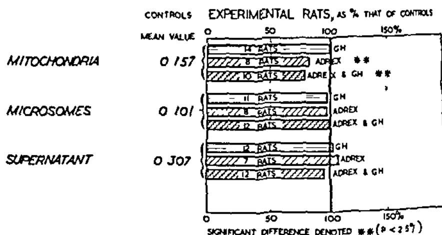


Fig 2 Data for female rats (as for Fig 1)

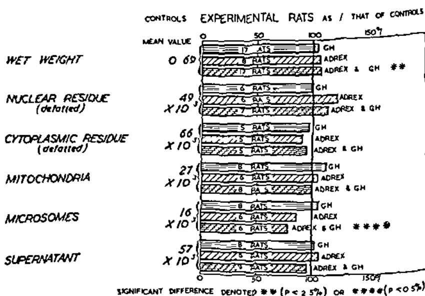


Fig 3 Data for female rats (as for Fig 1)

effect of hypophysectomy in reducing kidney weight was partially although not completely, reversed by GH No analyses on the kidney fractions for nucleic acid or other constituents have so far been performed

It was noted that hypophysectomy affected the relative weights of two other organs—the heart which was diminished by 10% and the lungs which were increased by 50% These effects which were not modified by GH treatment suggest that analysis of the composition of these organs would be of interest

The remaining data to be presented deal only with the liver With the kind collaboration of Dr A L Greenbaum and Mr T F Slater of University College London counts were performed under dark ground illumination on mitochondrial fractions freshly isolated from liver homogenates (*Table 1*) *The number of particles was not significantly altered by adrenalectomy* or hypophysectomy but a striking increase was observed in hypophysectomized rats given GH

Table 1

COUNTS ON SUSPENSIONS OF LARGE PARTICLES (MITOCHONDRIA) ISOLATED FROM RAT LIVER

Hormonal Status	*Particle Number * as % of that for control rats studied simultaneously (values calc as no /100 g body wt)*
Hypophysectomized	123 ± 17 5 (n =5)
Hypophysectomized + GH	371 ± 25 7 (n = 6 *P*<0 1%)
Adrenalectomized	116 ± 39 0 (n = 5)

* Mean ± s e n denotes no of observations and *P* the probability that difference from controls could be due to chance

Analyses on the Liver Cytoplasmic Fractions

The data for the hypophysectomized rats are conveniently considered in conjunction with those for the adrenalectomized rats (Fig 7) The data for the GH treated female rats whether intact or adrenalectomized showed no notable effect of the GH and will not be presented here Samples of each freeze dried fraction were treated with cold trichloracetic acid solution and then with lipid solvents to extract the lipid P the residual P was assumed to be entirely RNAP an assumption which cannot be seriously in error

As shown in Figure 7, the only significant change in lipid P was a diminution observed in the microsome fraction from hypophysectomized rats given GH But the RNAP analyses showed some striking changes The RNAP levels in the mitochondrial and microsome fractions were reduced in hypophysectomized rats and especially in the case of the microsomes in hypophysectomized rats given GH On the other hand the RNAP in the supernatant fraction was markedly elevated in GH treated hypophysecto-

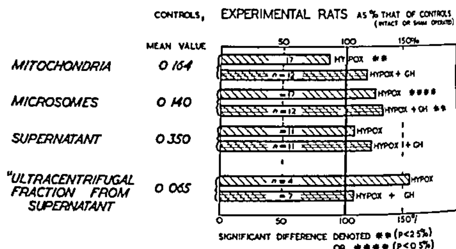


Fig 5 Data for male rats (as for Fig 4)

In the case of kidney (Fig 6) there was no significant effect of hypophysectomy or of GH on the yields of the freeze dried cytoplasmic fractions The hypophysectomized rats did however show a decrease in relative kidney weight as previously noted in Gaebler s laboratory [9] and also decreases in the yields of the defatted nuclear and cytoplasmic residues The

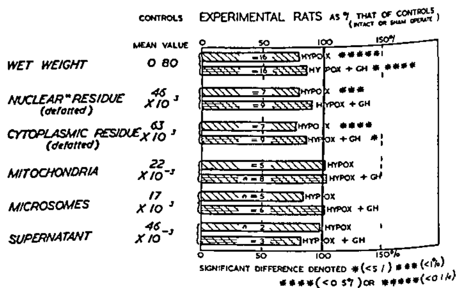


Fig 6 Data for male rats (as for Fig 4)

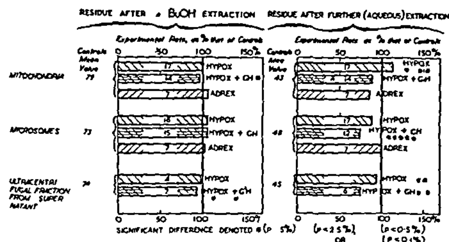


Fig 8 Data for male rats (as for Fig 7)

the mitochondrial fraction but decreased that from the microsome fraction With GH treatment the yields after aqueous extraction were consistently lower than with hypophysectomy alone but with adrenalectomy alone there were no significant changes

Analyses of the aqueous extracts for total N amide N and total P have shown an elevation of extractable P with the mitochondrial fraction from adrenalectomized rats and an elevation of extractable N with the microsome fraction from adrenalectomized rats given GH Although these were the only significant effects observed the same tendency to an increased extractability of mitochondrial P and of microsome N was evident with all three treatments studied—adrenalectomy GH treatment in intact rats and GH treatment after adrenalectomy No comparable analyses have so far been performed for the hypophysectomized rats used in the latter part of this study

Conclusions

The principal findings from the liver studies are summarized in Figure 9 Although GH can reverse the effect of hypophysectomy in diminishing the mitochondrial yield the fall in yield likewise observed with adrenalectomy suggests the involvement of ACTH also with an action similar to that of GH It will be recalled that a fall in the mitochondrial RNAP concentration was observed with hypophysectomy (with or without GH) but not with adrenalectomy accordingly the mitochondrial RNAP re-calculated in terms of body weight (Fig 9) shows a marked fall in the case of hypophysectomy but not of adrenalectomy

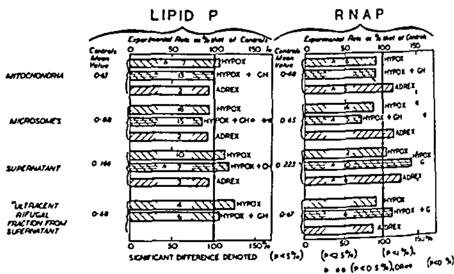


FIG 7 Data for male rats (effect of hypophysectomy and of GH) and for female rats (effect of adrenalectomy)

mized rats and in adrenalectomized rats although not in untreated hypophysectomized rats These findings suggest the possibility that the supernatant RNAP is affected not only by GH but conversely by ACTH This effect of GH is not exerted predominantly if at all on the fraction isolable from the supernatant by ultracentrifugation

So as to obtain aqueous extracts suitable for study of the protein composition by paper electrophoresis portions of the mitochondrial microsome and ultracentrifugal fractions were treated with *n* butanol at room temperature[1] and the residue extracted with borate buffer (Fig 8) With regard to the electrophoretic analysis of these extracts and of the supernatant fractions I need only say that crude comparisons of the protein patterns as revealed by finally dyeing the filter paper and eluting the protein bound dye have shown no consistent hormonal effects on the protein composition of any of the cytoplasmic fractions This aspect does however warrant a more refined study which should include analyses for nucleic acid the bulk of which ran ahead of the protein under the electrophoretic conditions employed (pH 8 3)

Some interesting hormonal effects have emerged from the actual extraction data (Fig 8) In the case of the mitochondrial and ultracentrifugal fractions the yield of residue from the butanol extraction was slightly diminished in the hypophysectomized rat given GH, a finding which suggests that the lipid content of these fractions was elevated Moreover hypophysectomy increased the yield of water insoluble residue from the further extraction of

lectomy have no significant effect on the particle number but that administration of GH to hypophysectomized rats leads to an increase greatly exceeding that in the weight yield It would appear that there is a pituitary hormone other than ACTH which has an action converse to that of GH on the particle number in the mitochondrial fraction

Some pituitary hormone other than ACTH may likewise influence the yield of residue obtained on aqueous extraction of the microsome fraction Here there is a fall after hypophysectomy which is actually enhanced by GH but no fall after adrenalectomy But in the case of mitochondrial extractability GH could be the only factor concerned since the decrease in extractability after hypophysectomy can be reversed by GH

The complex picture emerging from these various analyses is not readily interpretable especially in relation to current views on protein synthesis which is held to occur particularly in the microsome fraction[4 5 6] (*inter alia*) and to depend in some way on RNA[8 11] (*inter alia*) and possibly on phospholipids[13] If administration of GH to the hypophysectomized rats indeed led to increased protein synthesis in the liver—a conclusion admittedly contrary to that suggested by Bartlett and Glynn —then one could reasonably have expected such treatment to elevate liver RNA and this to be found in the microsome fraction The contrary is the case for it is in the supernatant fraction that we find an increase in the mitochondrial fraction and in the ultracentrifugal fraction there is no marked increase while in the microsome fraction there is definitely a decrease It is true that in studying protein synthesis Siekevitz[4] found it advantageous to use an enriched microsome fraction isolated pH 5 This pH 5 centrifugation in contrast with the ultracentrifugation now performed leaves no RNA in the supernatant and one wonders if it entails aggregation of material which was scarcely particulate It would be unprofitable to discuss the definition of the term microsome Evidently it is by no means proved that the shifts in liver RNA now observed have some significance unconnected with changes in protein synthesis

The absence of changes in phospholipid concentration other than a decrease in the microsome phospholipid in GH treated hypophysectomized rats is in general agreement with the findings reported by Geschwind Li and Evans[14] for whole liver The rate of incorporation of P into liver phospholipid is however known to be reduced by hypophysectomy and restored by GH[13 14] It would clearly be of interest to know in which cytoplasmic fraction or fractions these metabolic changes occur

Clearly there is a need for further study of hormonal influences on rat liver with particular reference to the distribution and also the composition and metabolic turnover of nucleic acid This study should include nuclear RNA since this has a particularly high turnover rate and since spectrophotometric studies[2] have shown that the effects of hypophysectomy and of GH are exerted on nuclear RNA as well as on cytoplasmic RNA

SUMMARY OF HORMONAL EFFECTS

O DENOTES NO SIGNIFICANT DIFFERENCE FROM INTACT (OR SHAM OP) CONTROL R DENOTES REVERSAL OF HYPOPHYSECTOMY EFFECT

DATA AS % OF BODY WEIGHT

		HYPOX	HYPOX + GH	ADRX
WHOLE LIVER	WET WEIGHT	↑	↑	O
	DNAP	O	O	↑
	RNAP	↓	O	O
MITOCHONDRIA	WEIGHT YIELD	↓	O(R)	↓
	NUMBER	O	↑	O
	RNAP	↓	O	O
MICROSOMES	WEIGHT YIELD	↑	↑	O
	RNAP	O	↓	O
SUPERNATANT	WEIGHT YIELD	O	O	O
	RNAP	O	↑	↑

DATA AS % OF FREEZE DRIED FRACTION

		HYPOX	HYPOX + GH	ADRX
WHOLE LIVER				
MITOCHONDRIA	RNAP	↓	O	O
	RESIDUE AFTER EXTRACTION BuOH	O	↓	O
	RESIDUE AFTER EXTRACTION AQ	↑	α	O
MICROSOMES	RNAP	↓	↓	O
	RESIDUE AFTER EXTRACTION BuOH	O	O	O
	RESIDUE AFTER EXTRACTION AQ	↓	↓	O
SUPERNATANT	RNAP	O	↑	↑

FIG 9 Data for male rats (as for Fig 7)

With the microsome fraction the net effect of the rise in yield and fall in RNAP concentration following hypophysectomy is that there is no change in total microsome RNAP in terms of body weight But in the case of hypophysectomized rats given GH the fall in microsome RNAP concentration outweighs the rise in microsome yield so that there is an actual fall in total microsome RNAP No consistent effect of adrenalectomy has emerged from the few analyses so far performed with microsome fractions

The total RNAP in the supernatant fraction is markedly raised both in adrenalectomized rats and in GH treated hypophysectomized rats The absence of any change in untreated hypophysectomized rats is readily explicable if the RNAP of the supernatant fraction is influenced in converse directions by both GH and ACTH But it is paradoxical that cortisone given to adrenalectomized rats tends to increase rather than diminish the supernatant fraction RNAP Reference should be made to results obtained by Lowe and Williams [14] who subjected the livers of cortisone treated intact rats to differential centrifugation in saline In these rats given cortisone acetate at the heroic dosage of 25 mg/day there was virtually complete deletion of mitochondria and microsomes or at least of the RNA in these fractions On the other hand there was a marked rise in the RNA of the supernatant fraction and particularly of the ultracentrifugal fraction Their results may be of no physiological significance but are of interest as showing an effect on the supernatant fraction somewhat similar to those now observed in adrenalectomized rats with or without low cortisone doses

The mitochondrial counts have shown that hypophysectomy and adrena

Mr James Salter has been doing in my laboratory and (2) aspects of the work which Dr Otakar Sirek another of my graduate students has just completed

Dr Lukens has already referred to the effect of insulin on completely hypophysectomized rats and to the two papers which have been published from our laboratory on this subject After a very brief review I will mention



Fig 2 The photomicrograph on the left represents a section of the epiphyseal disk from the tibia of an untreated hypophysectomized rat Cessation of growth is indicated by marked narrowing of the disk and by the extensive replacement of the metaphysis with marrow

The photomicrograph on the right shows the stimulating effect of insulin on bone growth in the hypophysectomized rat The epiphyseal disk is much wider than that of the control and shows a marked increase in the number and size of proliferating cartilage cells In contrast to the control the metaphysis is wider and good bone growth is evident upon the degenerating cartilage spicules (See also Salter J M and C H Best *Brit Med J* 2 353 (1953))

References

1 Geschwind I Li C H and H M Evans *Arch Biochem* 28 73 (1950)
2 DiStefano H S Bass A D Diermeier H F and J Tepperman *Endocrinology* 51 386 (1952)
3 Reid E *Cancer Research* 14 249 (1954)
4 Siekevitz P *J Biol Chem* 195 549 (1952)
5 Zamecnik P C and E B Keller *J Biol Chem* 209 337 (1954)
6 Alfrey V Daly M M and A E Mirsky *J Gen Physiol* 37 157 (1953)
7 Schneider W C *J Biol Chem* 176 259 (1948)
8 Smith H Keppie J and J L Stanley *Brit J Exp Pathol* 34 477 (1953)
9 Bartlett P D and M Glynn *J Biol Chem* 187 261 (1950)
10 Morton R K *Nature* (London) 166 1092 (1950)
11 Koritz S C and H Chantrenne *Biochem Biophys Acta* 13 209 (1954)
12 Lowe C U and W L Williams *Proc Soc Exp Biol Med* 84 70 (1953)
13 Cornatzer W E Gallo D G and J P Davison *Proc Soc Exp Biol Med* 84 103 (1953)
14 Geschwind I Li C H and H M Evans *Endocrinology* 47 162 (1950)

DISCUSSION

Growth Hormone and Energy Sources

Designated Discussion

CHARLES H BEST (University of Toronto) For this ten minute discussion which I am listed to give I have been uncertain whether I should comment on the previous papers or introduce some new material Fashion favours the latter course and I will attempt a brief description (1) of the work which

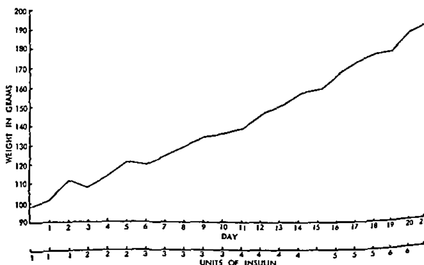


Fig 1 Weight changes in a hypophysectomized rat induced by the daily administration of insulin The weight of the animal remained constant during a five month period prior to insulin treatment (See also Best C H *Diabetes* 1 266 (1952) Salter J M and C H Best *Brit Med J* 2 353 (1953))

only much heavier but it is actually longer The increase in weight and size of the animal is quite obvious and it has been shown in comparable animals that this is due to an increase in fat protein and water content of the body

I will turn now to more recent work Mr Salter who is here today has been working on Houssay rats i e completely hypophysectomized animals which have subsequently received large doses of alloxan There was a preliminary loss in weight and these alloxanized animals were constantly glucosuric in spite of the absence of the pituitary After five days 5 animals were given insulin alone 5 were given growth hormone in doses ranging from 100 to 300 gamma per day and another group of 5 acted as controls The insulin treated animals began to gain weight and the slope of this weight gain increased as the dose was augmented No increase in the weight of the growth hormone treated animals resulted until they received insulin as well as the growth hormone and following this i e after the eleventh day there was a steep rise in body weight The rate of gain was a little faster than the insulin treated animals exhibited at this particular phase Both groups of animals were receiving the same dose of insulin i e the dose of growth hormone at this stage was superimposed on that of the insulin which the other group received

Another point which may be introduced is that hypophysectomized rats when given thyroxin increase in weight The first reference to these observations of our laboratory was made by Professor Leblond some six months ago in *Endocrinology* The details have not been published yet but a paper by Salter and Melgaard will go to press in the near future The first report on this positive finding with thyroxin in hypophysectomized animals i e the first convincing positive report was made by Scow in a recent number of *Endocrinology* Our findings are essentially similar This gain in weight with thyroxin is due to an increase of all the body constitutents i e fat protein and water and there is a definite increase in the width of the epiphyseal line Professor Evans and his colleagues have already shown a marked synergism between the growth effects of thyroxin and pituitary growth hormone an aspect which Dr Geschwind discussed yesterday In the three groups of animals the greatest increase in weight is in the insulin and thyroxin group the intermediate group is insulin thyroxin and cortisone the group which shows a slightly smaller increase is the one with insulin alone Interestingly enough the food intake of the group receiving insulin thyroxin and cortisone is considerably greater than the ones receiving insulin and thyroxin although the growth rate of the latter was appreciably greater The rats treated with insulin and thyroxin synthesized protein at a very rapid rate as judged by the total body protein The average increase in total body protein of these hypophysectomized animals over the controls was approximately 8 grams in the fifteen or sixteen days of the experiment

The work which Dr Otakar Sirek has been doing under my general supervision during the last three years may be summarized very briefly as

some of our more recent findings In Figure 1 the growth curve of a completely hypophysectomized rat is shown The animal had been hypophysectomized several months prior to treatment and had not grown at all This slide is shown to dispel the illusion that a profound increase in weight does not result when insulin alone is given to a completely hypophysectomized animal In three weeks this animal almost doubled its weight The increase in width of the epiphyseal cartilage is shown in Figure 2 where the width is almost twice that of the untreated control animals The figures for nitrogen retention have been published and need not be mentioned in detail here In Figure 3 a control and test rat both hypophysectomized are shown above and below on the right the insulin treated animal This animal is not

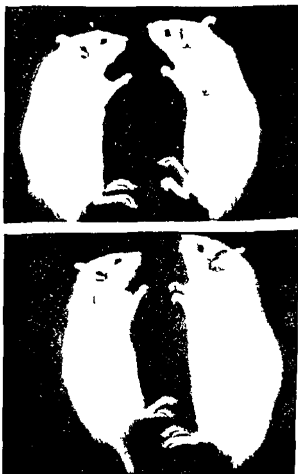


FIG 3 The upper photographs show two untreated hypophysectomized rats of the same size and weight

The two lower photographs show the same animals 30 days later During this latter period the rat on the right has received daily injections of protamine zinc insulin and shows a marked increase in weight and size

in stimulating the oxidation of carbohydrate but the energy thus made available is used in part for anabolic purposes It restores growth to the young diabetic organism just as thyroid hormone for example reestablishes this process in the thyroidectomized animal There is an abundance of data suggesting that one of the actions of somatotropin is to directly or indirectly liberate insulin Considerable further support for this mechanism probably will be provided at this meeting I was not convinced by Dr Lukens sugges tion that the action of insulin is transient It could be that the effect of sugar on the liberation of insulin is a transient one while the effect of growth hor mone on the same system is less transient Whatever the answers it is obvious there is a great deal to be learned of the interactions of the various growth hormones

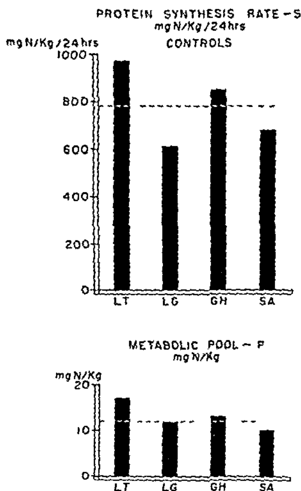


FIG 4

follows He has been studying depancreatized dogs i e animals from which all the pancreas has been removed These dogs are treated with insulin until they are in good shape at which time the insulin is withheld for 24 or 72 hours and the response of the animals to growth hormone and to insulin or to the two together is measured The blood sugar and blood amino acids have been followed In diabetic dogs 24 hours without insulin the growth hormone may produce a rise or a fall in blood sugar and the direction of this seems to depend on the initial level of blood sugar It will be appreciated that the level of blood sugar 24 hours after insulin is withdrawn can be readily controlled by the amount of insulin given on the previous day When the blood sugar on the experimental day is high i e well above 100 mg per cent there is a significant fall following the administration of growth hormone When the blood sugar is relatively low there is a rise in the level after the administered growth hormone These findings are in extension of those of our friend de Bodo The amino acid content of the blood of these animals consistently falls irrespective of the level of the blood sugar Dr Sirek has found that the rise in blood sugar can be completely eliminated when the adrenergic system is blocked by dihydroergotamine These findings indicate therefore that this early rise is associated with the action of epinephrine It will be remembered that in the normal dog growth hormone does not cause any rapid change in blood sugar level but the blood amino acid concentration falls In diabetic dogs 72 hours after the last dose of insulin growth hormone produces a much smaller fall in blood sugar level and no change in that of the amino acids If these diabetic dogs are pre treated with growth hormone (the dose has always been 12 milligrams per kilogram of body weight and given in two injections) there is no change in both blood sugar and amino acid concentration 24 hours after the last dose of insulin A fourth point is that animals given *insulin* 24 hours after the previous dose show a fall in both sugar and amino acid concentrations as would be expected if given *growth hormone* they have a fall in the blood level of sugar and amino acids But the point we wish to make here is that if the growth hormone is added to the dose of insulin i e the two given together there has not been any augmentation of the effect produced by either substance alone

The general conclusions from this work are that a certain amount of residual insulin is necessary for the protein anabolic effect of growth hormone as judged by the fall of blood amino acids and for the utilization of sugar as judged by the fall in blood sugar Dr Sirek will continue with this type of work on Houssay dogs and Mr Salter will concentrate for a time on the growth effects of the various hormones in Houssay rats

Insulin is one of several growth promoting hormones It has a variety of anabolic effects which favour the synthesis of protein fat and carbohydrate It stimulates the growth of bone epiphyses (as Dr Honor Fell has told us) and of cells in the chick heart explant (Dr Leslie) It has a catabolic effect

in stimulating the oxidation of carbohydrate but the energy thus made available is used in part for anabolic purposes It restores growth to the young diabetic organism just as thyroid hormone for example reestablishes this process in the thyroidectomized animal There is an abundance of data suggesting that one of the actions of somatotropin is to directly or indirectly liberate insulin Considerable further support for this mechanism probably will be provided at this meeting I was not convinced by Dr Lukens suggestion that the action of insulin is transient It could be that the effect of sugar on the liberation of insulin is a transient one while the effect of growth hormone on the same system is less transient Whatever the answers it is obvious there is a great deal to be learned of the interactions of the various growth hormones

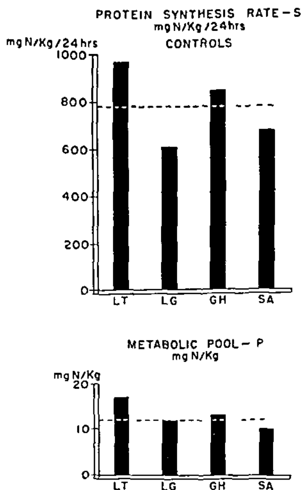


Fig 4

General Discussion

KENNETH CRISPELL (University of Virginia School of Medicine) Dr William Parson Dr Guy Hollifield and I have been using the N[15] tagged glycine technique as described by San Pietro and Rittenberg to study amino acid metabolism in patients with various types of endocrine disorders This is the same technique that Dr Bartlett used in his dogs

We first studied four healthy medical students on a standard metabolic regime and found the size of the amino acid pool to average 12 mg of nitrogen per kg body weight (range 10–17 mg) and the protein synthesis rate to average 780 mg of nitrogen per kg body weight per 24 hours (range 610–970 mg) Data from this study are shown in Figure 4

We then studied a 20 year-old pituitary dwarf This boy at age 8 was operated upon for a suprasellar tumor and a large mount of pituitary tissue was removed This dwarf had not received hormonal therapy at any time and was neither perceptibly growing nor maturing

Dr Maurice Raben kindly supplied us with a growth hormone preparation from his laboratory After a two week balance period in which the dwarf was in nitrogen equilibrium on a diet containing one gram of protein per kilogram of body weight he was given injections of growth hormone 20 mg every six hours for six days

Figure 5 will bring out the following points

1 The growth hormone altered nitrogen balance somewhat erratically

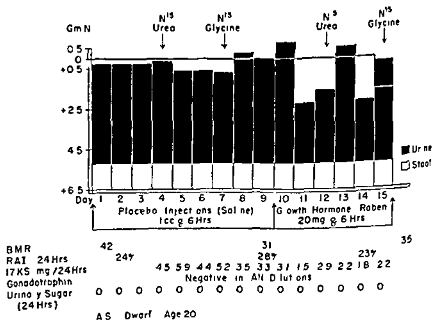


FIG 5

However during the six days of growth hormone there was a total nitrogen (N) retention of 2 0 g as compared to the six day control period

2 There was no evidence of adrenal or thyroid stimulation at least as could be determined by increases in 17 ketosteroid excretion in radioactive iodine uptake or in basal metabolic rate

3 There was no glycosuria at any time he was receiving growth hormone

4 The N^{15} studies were carried out in the control period and during the *administration of growth hormone as indicated on the chart On the 'tag* day while the patient was receiving growth hormone it will be noted urinary N was the same as on the tag day during the control period Actually on

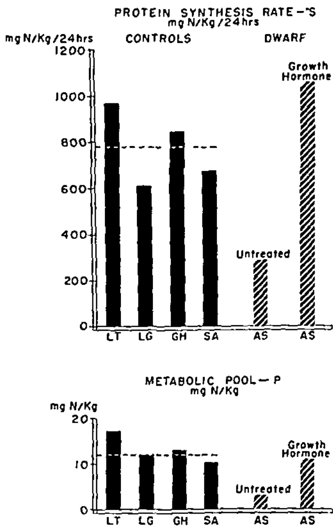


FIG 6

the tag day of growth hormone administration the patient was in negative balance because nausea prevented him from taking the bedtime feeding. As you see his 24 hour intake decreased

In Figure 6 is shown the markedly decreased synthesis rate as mg nitrogen/kg /24 hours for the dwarf as compared to that of the normals. The size of the metabolic pool is also markedly decreased Note that after the administration of growth hormone to the dwarf there was a marked increase in protein synthesis rate and in the size of the metabolic pool This was not due to any alteration in the size of the urea pool which was the same on both occasions

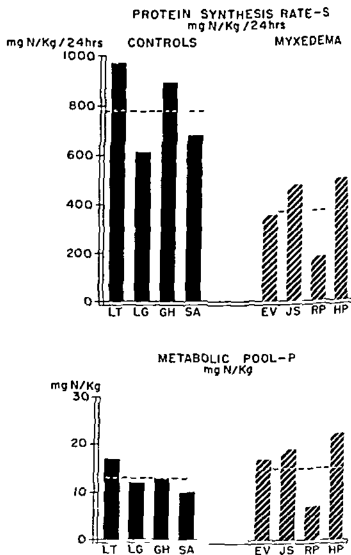


FIG 7

Now as seems to be the order of the day we have demonstrated on one occasion the growth hormone effect but have failed in the next three attempts Since this first patient we have studied another with pituitary dwarfism a patient with hypoadrenalism and diabetes mellitus and finally a patient with hypopituitarism due to post partum pituitary necrosis The last patient was studied while she was being maintained on cortisone In none of these patients have we been able to show any evidence of growth hormone effect either by classical nitrogen balance methods or by this isotope technique However we have not lost faith completely and will continue in our attempts to show that growth hormone can produce changes which are measurable by this technique

Next one might ask why should we use this complicated technique if it does not give any more information than can be obtained from the classical nitrogen balance studies We feel that similar isotope studies in patients with myxedema have given information not supplied by the more traditional balance studies Four patients with advanced myxedema were found to be slightly in nitrogen storage when the usual balance technique was employed yet all four patients were found to have decreased protein synthesis rates when their data were compared to those of the controls (see Fig 7)

We have recently completed similar studies on one of these patients in whom the euthyroid state had been obtained by the administration of L triiodothyronine There was a profound change in the synthesis rate from a pretreatment level of 350 mg of nitrogen kg /24 hours to a treatment level of 1300 mg /kg /24 hours

Perhaps this isotopic technique will prove to be a way by which the overall protein synthesis rate for the whole organism can be studied We feel that it warrants continued study

ERNEST KNOBIL (Harvard Medical School) I would like to continue in the frame of reference initiated by Dr Wilhelmi In his paper of yesterday he illustrated quite plainly certain species differences with respect to the responsiveness to a given growth hormone preparation To be more specific he mentioned the fact that the fish responded to both fish and beef growth hormones while the rat responded to beef growth hormone and not to that of fish Dr Greep and I have studied the effects of growth hormone in normal and hypophysectomized rhesus monkeys We have administered various growth hormone preparations chronically at the rate of 5 mg per kg per day over prolonged periods of time We have studied also the acute effects of intravenously administered growth hormone To make a long story very short we have been unable to demonstrate any growth promoting effects in beef or pig pituitary growth hormones when the preparations were administered to either normal or hypophysectomized monkeys over a period of several weeks Likewise we have been unable to demonstrate unequivocal

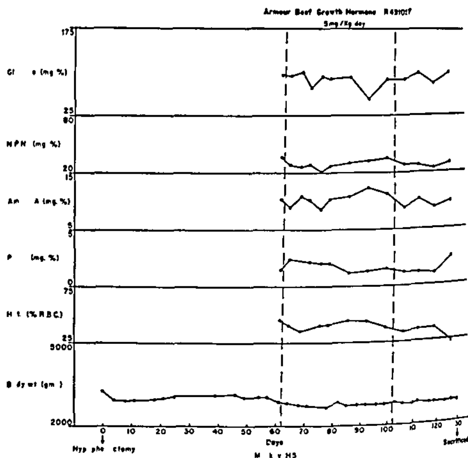


FIG 8

changes in blood levels of amino acid nitrogen non protein nitrogen glucose inorganic phosphorus and other blood constituents In Figure 8 is illustrated one of our chronic experiments in a hypophysectomized rhesus monkey The initial NPN levels in this particular animal were low and they were unchanged by 5 mg per kg per day of an Armour beef growth hormone prepared by the Wilhelmi procedure Amino acid nitrogen also did not change significantly during the course of hormone administration The variations during therapy were within the limits of normal day to day changes as observed in our animals Inorganic phosphorus levels remained unaffected as you can see Body weight certainly was uninfluenced by this course of growth hormone administration In Figure 9 is demonstrated the effect of a very large intravenous dose of growth hormone in two normal anesthetized monkeys which were studied over a period of some 8 hours In this experimental procedure growth hormone (the solid line) did not bring about any greater changes than were observed in the control animals given an equal volume of saline We realized the limitations of this type of an experiment and

EFFECT OF GROWTH HORMONE ON BLOOD AMINO NITROGEN
20 mg / Kg IV (ARMOUR LOT No R491017)

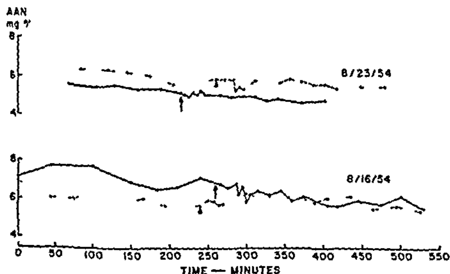


Fig 9

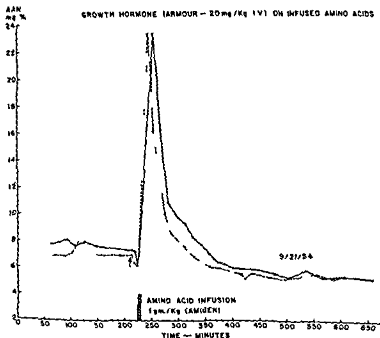


Fig 10

decided to administer intravenously an amino acid mixture casein hydrolysate In Figure 10 are shown the effects we observed when casein hydrolysate (Amigen®*) was administered intravenously to an animal pretreated by growth hormone (the solid line) while a control animal received an intravenous injection of an equal volume of saline (the dotted line) It will b noted that the blood amino acid nitrogen rose to the same level in both animals Unexpectedly however the blood amino acid nitrogen concentrations in the growth hormone treated animals took a longer time to return to normal levels than did those of the control animals In Figure 11 are shown our observations on blood inorganic phosphorus levels and th effect of large doses of intravenously administered growth hormone No significant differences were observed between the growth hormone treated animals (solid line) and their controls (dotted line)

In considering the significance of data of this kind one must keep in mind some of the things which Dr Wilhelmi said yesterday There may be slight

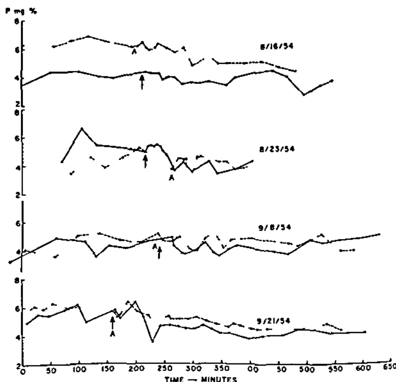


Fig 11

* Glucose free

chemical differences in the molecular structure of the growth hormone from the several species In addition there may be species differences with reference to the responsiveness to a certain growth hormone preparation We are now in the process with the kind cooperation of Dr McGinty of Parke Davis and Company of acquiring large numbers of monkey pituitary glands It is our hope that we can isolate enough monkey growth hormone to permit a repetition of these studies in a more direct attack on this particular problem

PHILIP BONDY (Yale University School of Medicine) There are one or two points I wish clarified I am not very familiar with the isotope techniques which Dr Bartlett and Dr Crispell used but it is my impression that the original mathematics proposed by Drs Sprinson and Rittenberg which later were extended by Dr Hoberman assumed a steady metabolic state Now Dr Crispell s observations on normal persons and Dr Bartlett s observations on plateaued dogs would fit this situation I am not sure however whether the observations on animals changing rapidly during starvation or after the administration of growth hormone are susceptible to the same type of mathematical analysis Would one of the authors be willing to briefly indicate whether or not this question has been raised in his mind? The other question is in regard to the changes in fluid volume of animals under treatment with growth hormone Dr Dougherty has already shown that there are changes of probably considerable degree in the blood volume of at least some animals receiving growth hormone It occurred to me that some of the concentration changes discussed by Dr Bartlett might have been due in part to this action of the hormone

PAUL BARTLETT In respect to your first comment about the possibility that some of our conditions were not truly steady metabolic states I can reply that we were well aware of this point before we made our experiments We attempted to get the animal at what would correspond to a steady state in respect to the effects of growth hormone i e we waited until the 4th or 5th days of growth hormone stimulation In our previous investigations with the normal adult dogs we had found that if we continued the administration of growth hormone beyond a 5 day period the animal invariably would start losing the nitrogen which it had stored Accordingly we feel that the animal at the 4th or 5th days has attained so to speak a new steady state in respect to protein synthesis amino acid catabolism and the size of the nitrogen pool

Now in respect to your second question concerning changes in fluid volume I must say we have not made measurements of plasma volume in these animal We have followed the hematocrit on occasion and in the few studies there were no marked changes You may recall perhaps that on the slides used in my discussion of the size of the urea pool data were presented

which concerned the size of the urea space and total body water as determined by the procedure of San Pietro and Rittenberg In these studies we found in one animal a slight decrease in total body water while in the other two animals a slight increase We were unable to draw any significant conclusions from these data

While I have this opportunity, I would like to make a few comments in connection with some of the papers which have been presented Lest you go away with the impression that we observed a decrease in liver weight in all hypophysectomized animals during growth hormone treatment I will hasten to say that these were experiments with pair fed animals That is we observed this decrease in liver weight of animals which were hypophysectomized, treated with growth hormone and pair fed with the untreated animals Therefore, their food intake was greatly restricted In these same animals we observed an increase in the muscle mass particularly that of one isolated muscle, the tibialis anticus Our explanation was that the animal was restricted in food intake and had, therefore a limited supply of the amino acid building blocks necessary for protein syntheses I believe it has been demonstrated repeatedly that muscle is the chief site of protein syntheses in both the hypophysectomized rat and the adult normal rat treated with growth hormone Accordingly I think it is well for all of us to take into consideration when we are measuring some metabolic component whether or not an organ weight is increasing at the same time In view of the well known observation of Addis and associates that the liver will break down its substance to supply amino acids for other sites of greater metabolic demand, it seemed likely that this was the explanation of the results in our experiments

The other comment which I wish to make is in connection with the size of the nitrogen pool In my presentation I purposely avoided mentioning the results of our rat work in view of the time limitations and presented only the results of our dog studies There is other evidence which seems to support the idea that growth hormone produces an increase in the metabolic pool of nitrogen and that this takes place chiefly in muscle I am referring to our data in a study of hypophysectomized rats treated with growth hormone In this study of some years ago we determined total free amino acids and glutamine in the tibialis anticus Total free amino acid nitrogen in this particular muscle was elevated and glutamine amide nitrogen was decreased during the period of nitrogen storage induced with growth hormone

BERNARDO A HOUSSAY (Institute of Biology and Experimental Medicine Argentina) In the hypophysectomized rat folic acid and vitamin B_1 enhance the action of growth hormone on the body weight and on the weight of liver, kidney and many other organs The action of folic acid is more

marked than that of vitamin B_{12} When folic acid or vitamin B_{12} are given alone there is no such action

In respect to Dr Russell s paper I would like to mention that hypophysectomy in dogs cats and rats results in an impaired retention of nitrogen On this point there is general agreement However the catabolism of rats and dogs is different under various conditions They are able to catabolize amino acids quite easily the amino acids which come from digestion are utilized well When there is a need to mobilize their own protein however there is a real difference In the fasting rat there is an increased catabolism of protein while in the fasting or diabetic dog there is a definite lowering of protein catabolism Then I must emphasize the difference in the hypophysectomized dog between utilization of amino acids which is normal and the mobilization of protein which is impaired

JANE RUSSELL Thank you Dr Houssay for bringing up these observations which I remember very well There is one point concerning the rate of nitrogen catabolism in the hypophysectomized animal which I didn t mention this morning but which I think many of us recall It is that atrophy of the adrenal cortex and thyroid are present some little time after hypophysectomy Now if the fasting nitrogen excretion of the hypophysectomized rat is measured some 2 or 3 weeks postoperatively one finds a slowing down of the nitrogen catabolism and an impairment of protein mobilization as in the dog Dr Houssay described The conditions to which I was referring this morning were those of the immediate postoperative period They were deliberately chosen so that this factor would be relatively less important and under these conditions we can see the opposite effect of growth hormone lack on nitrogen catabolism

There are only two points in connection with other papers which I wish to discuss Firstly Dr McHenry pointed out that there are theoretically only two ways in which nitrogen retention may be brought about in respect to energy sources i e either by an increase in the supply of energy for the purpose of nitrogen retention or by a decrease in the rate of catabolism through a change in metabolic efficiency so to speak There is another way which I think we should all remember This was pointed out very well in some data of F G Young which concerned the relative caloric value of protein in tissue and of fat in tissue The caloric value of protein itself as we all know is about 4 calories per gram but tissue protein which is always laid down with water to an extent of some 3 or 4 times its weight has a caloric value of about 1 calorie per gram of wet tissue The caloric value of fatty tissue is very nearly that of fat itself some 8 or 9 calories per gram so that the oxidation of 1 gram of body fat would permit energy wise the laying down of 8 or 9 grams of tissue containing protein and water in the usual proportions Thus there need not be any change in the food intake

or in the metabolic efficiency to allow an animal on restricted food intake to gain weight under the influence of growth hormone

Another point concerns some of Dr Bartlett s observations as they relate to my own He used some figures derived from Hoberman s calculations on isotope excretion to indicate a change in the rate of amino acid catabolism I think that was his interpretation The figures relate to the per cent of the nitrogen pool which is catabolized per unit time In the derivation of these equations Dr Hoberman assumed that the catabolism of amino acids was a first order reaction with respect to the size of the nitrogen pool and accordingly he compared the k s the constants of this first order reaction in variously treated animals There is no evidence however that the catabolism of amino acid is a first order reaction with respect to the nitrogen pool In fact some data of Sprinson and Rittenberg in their study of normal rats fed different amounts of protein indicate clearly that this is not the case Accordingly one cannot compare the rate of nitrogen catabolism as a fraction of the total nitrogen in the metabolic pool This fraction is nothing more than the ratio of the nitrogen excretion rate to the nitrogen pool and either one may vary independently With this in mind I think that these calculations have little validity in interpreting the rate of amino acid catabolism The effects of growth hormone on amino acid catabolism are evident in the drop in urea formation and excretion but diminished nitrogen excretion does not tell us that growth hormone slowed the catabolism of amino acids per se or that it altered either the breakdown or synthesis of protein

As one final point I wish not to leave the impression that I thought insulin had nothing to do with nitrogen retention or with growth hormone I certainly did not intend to imply that Very clearly it does have an effect There is a difference however between the action of insulin and that of growth hormone in the experiments which we and others have performed This difference is that insulin always requires the simultaneous administration of carbohydrate to bring about its effects Large amounts of insulin have been given in certain of the experiments which have been described particularly in those of Dr Best The addition of carbohydrate is one of the things on which the action of insulin is contingent On the other hand the action of growth hormone does not require more carbohydrate than the animal can make available from endogenous sources If we recall that difference we are not in disagreement as to the relative importance of insulin and growth hormone in nitrogen retention

PAUL BARTLETT The point raised by Dr Russell concerning the relationship of the rate of nitrogen catabolism (as represented by nitrogen excretion) to the size of the nitrogen pool is certainly important It is indeed true that if ratios of the total urinary nitrogen excretion per 24 hours to the total size of the nitrogen pool are calculated variations over wide range are obtained On this basis as Dr Russell has indicated the rate of nitrogen

catabolism (as represented by nitrogen excretion) is not directly proportional to the size of the nitrogen pool

Recently San Pietro and Rittenberg have shown that the rate of excretion of urea from the urea pool is a rate determining step which must be taken into consideration in the evaluation of the size of the metabolic pool of nitrogen Values for the size of the metabolic pool of nitrogen which were determined for rats maintained on diets of various nitrogen content were actually some unknown combination of the nitrogen pool and the urea pool In order to clarify the question raised by Dr Russell concerning the proportionality of the rate of nitrogen catabolism to the size of the nitrogen pool the experiments on rats receiving diets of varying nitrogen content should be repeated and the size of the metabolic pool of nitrogen be re-evaluated in terms of the kinetic refinements described by San Pietro and Rittenberg

T LEVITT As a result of this discussion I am slightly perplexed Are we led to believe that there is one comprehensive growth hormone emanating from the pituitary and affecting endocrine target organs like thyroid pancreas etc or are we to understand that these target endocrine organs may also produce growth hormones? I seek enlightenment on this problem

F D W LUKENS Dr Levitt we recognize that there are many hormonal factors which influence growth and we are struggling in a rather beginner s fashion as I see it to put them all together Now pituitary growth hormone is a very potent agent but it doesn t act alone on an isolated tissue or on one which has been deprived of all other hormones and nutritional factors The integration of these things as Dr Weiss pointed out previously is practically the whole field of biology We are just touching on a few items in that balance account of which he so graciously spoke

While I am here I might ask two questions This problem of species difference is always stimulating to biological research Since the rat is so often used in our studies I would like to ask whether anybody here and particularly Dr Wilhelmi knows of the growth hormone content of the rat pituitary It would make some difference as to what kind of a substrate this creature was Secondly I would like to ask Dr Best whether he has any comments on this following observation We could demonstrate a large utilization of glucose by giving 0 5 unit of insulin to Houssay cats which weighed more than 2 kilograms while his (Dr Best) dose of insulin went up to 3 units per rat If we arbitrarily accept 200 grams as being the rat weight and put all these figures together it appears that the hypophysectomized rat is 60 times as resistant to insulin as is the hypophysectomized cat Now that background may have some real bearing on the remarkable behavior of the hypophysectomized rat to insulin for it is responding to a much larger dose of insulin than we can use in the cat

ALFRED WILHELMI I am afraid there isn t any answer to the question of how much growth hormone there is in the pituitary gland of any species In order to find that out we need only to refer back to the first morning and the remarks about the many factors which can interfere with the assay of growth hormone in mixtures especially in crude mixtures Until we have a sensitive method and one which is indifferent to the actions of the other principals likely to be found in the pituitary soup (which would have to be used for the estimate in the whole pituitary), we must reserve our answer to this question I hope that we will find such a method for then we can begin to make proper and accurate comparisons not only between the pituitaries of different species, but also between the pituitaries of animals of the same species at different periods of their lives

CHARLES BEST There are two or three points I might mention Dr Renshaw from our department has made some calculations relating to the use of isotopes in dynamic disequilibrium which are rather interesting Dr Bondy might wish to look these up in the physical journals Dr Renshaw obtained his Ph D in mathematics and physics at Yale as a preliminary to tackling physiology in Toronto There is a lot more fun working on problems of dynamic disequilibrium than always trying to get a steady metabolic state

I should thank Dr Russell for her comments There is no difference in our conceptions of the relative actions of these various growth promoting hormones

I have no further comments on Dr Lukens work I was trying to search out some point of disagreement and to stimulate him to say something more about the liberation of insulin by carbohydrate and by pituitary growth hormone

Dr Honor Fell s work on the width increase in the epiphysis of bone was performed in chicks and not in rats so there is another species in which these changes can result from adding insulin to the medium I was thinking that if you had to restrict the bone substrate in the way that it is sometimes suggested we restrict carbohydrate intake of the hypophysectomized animals those bone cultures wouldn t grow either It seems unsound when you are using insulin to consider restricting the very substrate which it primarily uses

OSCAR RIDDLE In view of these very important statements concerning the role of insulin in growth and in view of what has been said this morning and perhaps yesterday which I didn t hear about differences in the action of these growth hormones on various species, there is something further to be said about the action in another species of still another pituitary hormone which acts both on insulin and on growth I ask you to remember some work which was published from our laboratory regarding prolactin which in the pigeon will produce more growth than will any other hormone or sub

stance Now it will do that in 7 or 8 days and it will do it mostly by causing the pigeon to eat 2 or 3 times more than it ordinarily eats When one restricts the diet of the pigeon normal or hypophysectomized to a little less than half its normal amount one finds growth occurring in certain regions Is it very restricted? Hardly It s splanchnomegaly which one sees in the pigeon under that reduced food intake Now this splanchnomegaly is found throughout the whole length of the gut, in the liver and in the pancreas When one fasts the normal or the hypophysectomized pigeon re ceiving prolactin one will not see a maintenance of growth beyond that seen in controls and which occurs in all of the splanchnic organs with the pos sible exception now of the pancreas Remember however that our work with Miller which was carried out under similar conditions and under complete fast revealed evident stimulation of the insulin secreting cells Therefore this newer work which indicates insulin is an adjuvant to growth hormone in supporting growth in certain species is parallel to the prolactin insulin situation in the pigeon studies This emphasizes again the im portance of species differences and the general role of insulin as an adjuvant Remember, too that the androgenic hormones are adjuvant for they add to growth in normal or hypophysectomized pigeons and that thyroxin adds to it just as it does in mammals

MILTON LEE (Chairman) Should I ask you whether the prolactin was at all pure?

OSCAR RIDDLE I wouldn t claim it was real pure but I will say that we tried to make sure that growth hormone was not present We did this but not by growth tests which we think others performed better Our laboratory never claimed proficiency in assaying growth hormone What we did however was to boil the growth hormone which was said to be heat labile for one hour at pH 8 0 or for 5 hours at 60 degrees Now perhaps you could tell me that growth hormone will stand this treatment There probably wasn t much of it in our prolactin preparations and in the tests which I just men tioned beta cell stimulation was found following the use of such prepara tions

MILTON LEE Your answer is quite adequate

PAUL WEISS I was very glad to hear Dr Riddle s comments because in this whole discussion the question of mutual competition of the organs during starvation or submarginal feeding has not been brought into adequate con sideration It is erroneous to think that if we cease feeding all the organs just stop at the level in which they are They go on to eat each other up at different ranking orders and I think this should be taken into consideration For that reason Dr Best s remark is very much to the point that is unless

you give something from the outside, the animal is going to get it some where from the inside and that will certainly disturb the picture

What I was getting up to ask was essentially that I was very glad that nucleic acids are being brought into the picture by Dr Reid It is one step in the desired direction However in order to emphasize the dangers inherent in these techniques—that is if you make mechanical determinations—I wish to ask a question perhaps a rhetorical question He said we will be confused by his results I have been somewhat confused but not enough so that I failed to note that there has been some system in the events Wherever a decrease occurred in either the mitochondrial or the microsomal fraction there was an increase in the corresponding parameter of the supernatant Now let us assume just for the sake of argument that these hormones or other applications have nothing to do with growth but change the fragility of these various cellular inclusions And if the mito hon dria or the microsomes are more fragile then obviously with the same degree of centrifugation more of them will explode and you will find their contents in the supernatant fluid I mean this effect is not a biological one It is a method perhaps of testing the fragility of such inclusions This example with its assumptions suggests certain of the dangers in a mere mechanical tally without attempting to determine what has happened in the experiment Moreover if the ribonucleic acids are increased I wish to repeat it tells us nothing about real growth being present since it may mean merely an increased activity of the gland of all glands or of the cyto plasm during which active state the ribonucleotides are rapidly multiplying Obviously no one will assume that the process of producing more insulin in the pancreas let us say is the same process which leads to the growth of a pancreatic cell such that it will give rise to two cells or more in hyper plasia and in hypertrophy Accordingly I would just like to point out once more that unless you pretend you are from Missouri should you not be from Missouri such determinations as these will likely lead you astray Nonetheless Dr Reid s study is the first step to a real penetration into the mechanics of what is going on during growth

MILTON LEE Dr Reid are you in agreement with those comments?

E REID I completely agree with the remarks of Dr Weiss and have chal lenged Dr DiStefano to make spectrophotometric determinations on individ ual mitochondria and microsomes but he didn t seem very willing to take up the challenge That step might answer the point

PHILIP J RANDLE (University of Cambridge London) We have heard from Dr Lukens that growth hormone promotes full nitrogen retention in depancreatized cats only if the dose of insulin is increased during the period of growth hormone administration We have heard from Dr Best also that

insulin can promote growth in hypophysectomized rats The question I would like to ask now is whether either of these authors thinks that the secretion of extra insulin as a response to growth hormone is a normal feature either in the rat or in the immature cat manifesting spontaneous growth I ask this because many years ago Young demonstrated that growth hormone—I should say crude pituitary extracts are not diabetogenic in the kitten or the puppy whereas they are in the adult cat and dog Subsequently Cotes Reid and Young demonstrated that growth hormone was diabetogenic in the adult cat and more recently returning to this problem in some preliminary experiments I have been unable to induce diabetes in the kitten with purified growth hormone Do Dr Lukens and Dr Best consider that extra insulin is secreted in a normally growing animal or in the rat and the kitten showing extra growth under the influence of administered growth hormone?

F D W LUKENS Yes I think extra insulin secretion probably does occur but we must realize that our measurements of this are indirect When we take out the pancreas and find that giving 3 or 4 times the maintenance dose of insulin produces something we infer there is an extra secretion of insulin in the intact animal showing a similar change But we certainly do not prove it in the sense in which your question was asked There is one clinical situation however which perhaps comes nearer to answering your question than anything else The young human diabetic goes through a period of normal growth quite satisfactorily provided the amount of insulin is adequate A few years ago I observed one of those unusual accidents or mistakes which can occur when a child with diabetes 8 years old was sent out of the hospital on 10 or 15 units of insulin a day Seven years later that child came back to us as a severe dwarf The insulin dose had not been increased during the critical period of his pubertal years However when the insulin dose was increased 4 fold to about 80 units per day the child grew about 4 inches in the next year reached an almost normal height and practically made up for the 7 lean years

CHARLES BEST I have nothing to add to what Dr Lukens has said except to say that the insulin content of course not an indication of insulin output is much greater in the pancreas of the young growing animal Indeed the commercial manufacturers of insulin are quite prepared to pay 3 or 4 times as much for calf pancreas as for adult beef pancreas

FRANK L ENGEL I was tremendously interested in Dr Russell s findings in the nephrectomized animals having carried out a similar study a few years ago in an attempt to localize the site of action of the adrenal hormones From the standpoint of considering the integration in the growth

you give something from the outside the animal is going to get it somewhere from the inside and that will certainly disturb the picture

What I was getting up to ask was essentially that I was very glad that nucleic acids are being brought into the picture by Dr Reid It is one step in the desired direction However in order to emphasize the dangers inherent in these techniques—that is if you make mechanical determinations—I wish to ask a question perhaps a rhetorical question He said we will be confused by his results I have been somewhat confused but not enough so that I failed to note that there has been some system in the events Wherever a decrease occurred in either the mitochondrial or the microsomal fraction there was an increase in the corresponding parameter of the supernatant Now let us assume just for the sake of argument that these hormones or other applications have nothing to do with growth but change the fragility of these various cellular inclusions And if the mitochondria or the microsomes are more fragile then obviously with the same degree of centrifugation more of them will explode and you will find their contents in the supernatant fluid I mean this effect is not a biological one It is a method perhaps of testing the fragility of such inclusions This example with its assumptions suggests certain of the dangers in a mere mechanical tally without attempting to determine what has happened in the experiment Moreover if the ribonucleic acids are increased I wish to repeat it tells us nothing about real growth being present since it may mean merely an increased activity of the gland of all glands or of the cytoplasm during which active state the ribonucleotides are rapidly multiplying Obviously no one will assume that the process of producing more insulin in the pancreas let us say is the same process which leads to the growth of a pancreatic cell such that it will give rise to two cells or more in hyperplasia and in hypertrophy Accordingly I would just like to point out once more that unless you pretend you are from Missouri should you not be from Missouri such determinations as these will likely lead you astray Nonetheless Dr Reid s study is the first step to a real penetration into the mechanics of what is going on during growth

MILTON LEE Dr Reid are you in agreement with those comments?

E REID I completely agree with the remarks of Dr Weiss and have challenged Dr DiStefano to make spectrophotometric determinations on individual mitochondria and microsomes but he didn t seem very willing to take up the challenge That step might answer the point

PHILIP J RANDLE (University of Cambridge London) We have heard from Dr Lukens that growth hormone promotes full nitrogen retention in depancreatized cats only if the dose of insulin is increased during the period of growth hormone administration We have heard from Dr Best also that

insulin can promote growth in hypophysectomized rats The question I would like to ask now is whether either of these authors thinks that the secretion of extra insulin as a response to growth hormone is a normal feature either in the rat or in the immature cat manifesting spontaneous growth I ask this because many years ago Young demonstrated that growth hormone—I should say crude pituitary extracts are not diabetogenic in the kitten or the puppy whereas they are in the adult cat and dog Subsequently Cotes Reid and Young demonstrated that growth hormone was diabetogenic in the adult cat and more recently returning to this problem in some preliminary experiments I have been unable to induce diabetes in the kitten with purified growth hormone Do Dr Lukens and Dr Best consider that extra insulin is secreted in a normally growing animal or in the rat and the kitten showing extra growth under the influence of administered growth hormone?

F D W LUKENS Yes I think extra insulin secretion probably does occur but we must realize that our measurements of this are indirect When we take out the pancreas and find that giving 3 or 4 times the maintenance dose of insulin produces something we infer there is an extra secretion of insulin in the intact animal showing a similar change But we certainly do not prove it in the sense in which your question was asked There is one clinical situation however which perhaps comes nearer to answering your question than anything else The young human diabetic goes through a period of normal growth quite satisfactorily provided the amount of insulin is adequate A few years ago I observed one of those unusual accidents or mistakes which can occur when a child with diabetes 8 years old was sent out of the hospital on 10 or 15 units of insulin a day Seven years later that child came back to us as a severe dwarf The insulin dose had not been increased during the critical period of his pubertal years However when the insulin dose was increased 4 fold to about 80 units per day the child grew about 4 inches in the next year reached an almost normal height and practically made up for the 7 lean years

CHARLES BEST *I have nothing to add to what Dr Lukens has said except* to say that the insulin content of course not an indication of insulin output is much greater in the pancreas of the young growing animal Indeed the commercial manufacturers of insulin are quite prepared to pay 3 or 4 times as much for calf pancreas as for adult beef pancreas

FRANK L ENGEL I was tremendously interested in Dr Russell s findings in the nephrectomized animals having carried out a similar study a few years ago in an attempt to localize the site of action of the adrenal hormones From the standpoint of considering the integration in the growth

process of the cortical hormones and the anterior pituitary growth hormone it might be worthwhile to comment very briefly on the differences between the actions of these hormones You will remember that Dr Russell considered the action of growth hormone to be essentially on amino acid metabolism In our study with the adrenocortical hormones we felt that their action was primarily on protein catabolism The action of the growth hormone is a rather rapid one while the action of the cortical hormones is delayed some hours in the nephrectomized animal Now, it is interesting that when we gave the adrenocortical hormone treated animal amino acids we could never demonstrate any increased urea formation This is in contrast to Dr Russell s findings of a decreased rate of urea formation following growth hormone When glucose was given to the nephrectomized animal which had received cortical hormone there was a marked dampening of the rise in urea formation expected with the adrenal hormone In other words we interpreted this as indicating a suppression by carbohydrate of the catabolism of protein We might contrast this with Dr Russell s findings that the administration of glucose *blocked out* the growth hormone effect on amino acids One might think, therefore, in terms of a balance since both these hormones are being secreted under normal circumstances With glucose we suppressed the protein catabolic effect of the adrenal hormone and thus decreased the need for the growth hormone anabolic effect in the presence of the glucose We administered fat emulsions and found absolutely no effect on the catabolic action of the cortical hormone In other words catabolism proceeded at its usual pace while Dr Russell finds that the nitrogen retaining effect is there in full force There, again we have a situation in which anabolism if it is going to act must *break through* so to speak the opposing forces And finally in the adrenalectomized animal we found that the infusion of amino acids had no effect on urea formation We did observe under similar circumstances that if the amino acids were infused with glucose in large amounts a rather sharp decrease took place in the rate of urea formation We wonder if in this instance we might have a determining point at which the adrenalectomized animal manifests the lack of catabolic influences from its own adrenal cortex while retaining his own growth hormone Accordingly an effect appears even though as Dr Russell recalled from her experiments with glucose no effect of the growth hormone was apparent It would be of interest to know what her comments might be on the difference between the metabolism of the adrenalectomized animal receiving both glucose and amino acids and that of the intact animal receiving the same

MILTON LEE It is important for us to participate in these discussions particularly to contribute a word or two on our own progress in these problems or to bring out certain discrepancies and warnings Dr Russell do you care to comment

JANE RUSSELL I am not quite sure that I followed all of the many comparisons which Dr Engel has just made although I am quite familiar with some of them

It is possible that the adrenalectomized animal does have a relative excess of growth hormone action One sometimes sees effects in the adrenalectomized animal which could be correlated with this view Such animals tend to have a higher concentration of protein in tissues as Winternitz and Long reported sometime ago and there are a number of other observations At the same time I am sure that growth hormone does not express the full potential of its action in the absence of the adrenal cortex

Now in answer to your last question it may be said perhaps that in the adrenalectomized animal the affect of glucose is actually on insulin glucose action and represents in part at least the inhibition by insulin of certain catabolic influences The inhibition of protein catabolism by insulin can be seen very well in the eviscerate animal No matter how much glucose one gives to an eviscerate animal one cannot effect the rate of release of amino acids from tissues unless insulin is given This can be called an anticatabolic action of insulin which may be what one sees in the adrenalectomized animal responding to glucose with an increased secretion of insulin But I am not sure that I have answered your question adequately

There is one little point I might bring up in connection with Dr Knobil s discussion on the effects of growth hormone in the monkey He reported that they were unable to see an effect of growth hormone on the disposition of the administered amino acids I may be in error but I think he said he used the commercial preparation of Amigen® in his studies * If this were the case it is unfortunate for all the commercial preparations of this material at least those which I have seen contained glucose One would not expect to see any effect of growth hormone under these conditions judged from our studies It would be necessary I think to repeat this particular experiment with an amino acid mixture free of glucose before concluding that no effect of growth hormone could be demonstrated

* In a post symposium communication Dr Knobil has indicated that the Amigen® preparation used in his studies did not contain glucose A footnote to this effect accompanies his discussion

16

Diabetogenic Actions of Growth Hormone

James Campbell
Department of Physiology University of Toronto Canada

It is my privilege to discuss the diabetogenicity of the growth hormone in this symposium and it is appropriate to begin with the question is the growth hormone diabetogenic? This question which has not yet been finally settled has been examined in the recent review by Young[1] The growth hormone isolated by Li Evans and Simpson was found to be homogeneous by electrophoretic and ultracentrifugal analysis and by its constant solubility Tests of biological activity indicated that the degree of contamination by other physiologically active constituents of the pituitary gland was of a low order Preparations of growth hormone which have been used in many biological studies appear to be contaminated by small amounts of other pituitary factors according to Ellis Noda Simpson and Evans[3] It does not seem possible however to ascribe the diabetogenic action of growth hormone to the small or trace amounts of thyroid stimulating hormone (TSH) adrenocorticotropic hormone (ACTH) prolactin and gonadotropins which the preparations may contain

The complete growth hormone molecule as isolated from the gland is not essential for full activity Two terminal residues of phenylalanine can be removed from the protein hormone by partial hydrolysis with carboxypeptidase without loss of growth activity (Harris Li Cardliffe and Pow)[4] No significant inactivation or change in the ratio of diabetogenic to growth activity was found by Reid when the growth hormone was treated with this enzyme the end groups alanine leucine lysine phenylalanine and serine were identified in the digestion mixture

The claim of Raben and Westermeyer[6] that growth hormone had been separated from diabetogenic activity has not been supported by subsequent studies (Reid[7] Houssay and Rodriguez)[8] The solubility of the Raben and Westermeyer product is low over a fairly wide range of pH on either side

of neutrality and Reid finds that the pH of the solution or suspension injected may alter the physiological response In our experience growth preparations made according to the technique of Raben and Westermeyer did not possess full growth promoting activity and did not elicit diabetes in the normal dog They did increase the sugar excretion and the blood sugar level of metahypophyseal (permanent pituitary) diabetic dogs that were given the usual dose of insulin but were less active in this respect than the growth hormone prepared according to Campbell and Davidson [9]

It must be acknowledged that until a naturally occurring product has been exhaustively characterized and has been synthesized chemically there will always remain a residuum of doubt as to its homogeneity It seems fair to state that the weight of evidence clearly favors the conclusion that the growth hormone is diabetogenic in the dog and the cat and this shall be accepted in the present discussion

The growth hormone is not the only diabetogenic substance of the anterior pituitary gland ACTH exerts a relatively slight diabetogenic effect of short duration in the dog when the amount injected is double the diabetogenic dose of growth hormone [10] ACTH does not cause glycosuria in the intact cat (Reid) [11] but it increases the excretion of sugar and nitrogen in the hypophysectomized depancreatized animal [1] The diabetogenic effect of growth hormone in cats is enhanced by the coincident injection of ACTH and apparently another enhancing factor present in certain anterior pituitary extracts but not of itself diabetogenic is required for this effect (Reid) [13] This co factor is present in the crude corticotropin fraction obtained from cattle and pig pituitary glands by the Raben and Westermeyer procedure According to Westermeyer and Raben[14] this fraction is rich in corticotropin intermedin and in adipokinin It inhibits the rise in the R Q after glucose administration has an anti insulin effect and lowers the blood sugar of mice The combination of growth hormone and ACTH injections in the rat force fed a high carbohydrate liquid diet elicited glycosuria but this result was not obtained when these hormones were given singly unless excessive amounts of the diet were force fed (Engel Viau Coggins and Lynn[15])

The presence of other pituitary hormones by themselves producing little or no diabetogenic effect may therefore modify the diabetogenicity of the growth hormone Because of these interferences the extent to which growth hormone participates in the total diabetogenic action of crude pituitary extracts cannot be estimated closely Tests on the diabetogenic activities of crude saline anterior pituitary extracts of partially purified fractions and of purified growth hormone separated from these extracts indicate however that a very large part of the total diabetogenic effect of the crude extracts can be attributed to the growth hormone [10] In respect to the property of eliciting diabetes in dogs therefore the growth hormone is the major diabetogenic substance of the anterior pituitary gland

16

Diabetogenic Actions of Growth Hormone

James Campbell
Department of Physiology University of Toronto Canada

It is my privilege to discuss the diabetogenicity of the growth hormone in this symposium and it is appropriate to begin with the question is the growth hormone diabetogenic? This question which has not yet been finally settled has been examined in the recent review by Young[1] The growth hormone isolated by Li Evans and Simpson was found to be homogeneous by electrophoretic and ultracentrifugal analysis and by its constant solubility Tests of biological activity indicated that the degree of contamination by other physiologically active constituents of the pituitary gland was of a low order Preparations of growth hormone which have been used in many biological studies appear to be contaminated by small amounts of other pituitary factors according to Ellis Noda Simpson and Evans[3] It does not seem possible however to ascribe the diabetogenic action of growth hormone to the small or trace amounts of thyroid stimulating hormone (TSH) adrenocorticotropic hormone (ACTH) prolactin and gonadotropins which the preparations may contain

The complete growth hormone molecule as isolated from the gland is not essential for full activity Two terminal residues of phenylalanine can be removed from the protein hormone by partial hydrolysis with carboxypeptidase without loss of growth activity (Harris Li Cardliffe and Pow)[4] No significant inactivation or change in the ratio of diabetogenic to growth activity was found by Reid when the growth hormone was treated with this enzyme the end groups alanine leucine lysine phenylalanine and serine were identified in the digestion mixture

The claim of Raben and Westermeyer[6] that growth hormone had been separated from diabetogenic activity has not been supported by subsequent studies (Reid[7] Houssay and Rodriguez)[8] The solubility of the Raben and Westermeyer product is low over a fairly wide range of pH on either side

the rate of removal The rate of formation of albumin was also increased by the hormone

The increase in plasma volume caused by the growth hormone can probably be attributed to the increased amounts of protein entering the plasma It can be expected that the extra amounts of protein will increase the protein osmotic pressure creating an osmotic imbalance between the plasma and the intercellular fluids and that to compensate for this the plasma volume will be increased

Within a day of the first injection of the growth hormone it is observed that the erythrocyte sedimentation rate (ESR) is increased and as the concentration of fibrinogen rises the ESR may rise to very high values It is well known that in other conditions an increase in the concentration of fibrinogen and of other large asymmetric molecules in the plasma causes a tendency towards aggregation of the red blood corpuscles and consequently an increase in the rate of sedimentation [1] The increased ESR caused by the growth hormone is probably due therefore to the increased concentrations of fibrinogen in the plasma

Increased white blood cell counts are observed within a day of the first injection of the growth hormone and in a few days marked leucocytosis is present [19] The polymorphonuclear neutrophil cells increase to the greatest extent The band cells increase and the monocytes also tend to increase in number The numbers of the other leucocytes i e lymphocytes eosinophils and basophils do not change significantly although as per cent of the total these cells decrease due to the increase in total cells

While these changes appear the blood sugar may remain within normal limits or may increase from day to day but not to diabetic levels In about 2 to 5 days of the injection period the concentration of sugar in the blood increases sharply and may rise to 300 mg % or more Ketonaemia follows the rise in blood sugar and shortly afterwards lipaemia is signalized by milky plasma in the morning blood samples Polyuria and polydipsia may precede the rise in blood sugar and the glycosuria Sugar appears in the urine and increases in amount for a few days about 50 to 100 grams may be excreted daily Ketonuria generally follows the glycosuria during the injections The excretion of total nitrogen and of urea is reduced during these injections while the excretion of uric acid is increased

The growth hormone in small doses increases the resistance of hypophysectomized dogs to insulin and changes their reaction to glucose so that a diabetic type of glucose tolerance curve is obtained (de Bodo Kurtz Ancowitz and Kiang) It is likely that analogous effects may be produced in normal dogs by the larger doses of growth hormone

Histological studies by Dr W S Hartroft and Mr W Wilson showed that in the intact dogs given growth hormone extensive degranulation of the beta cells of the islets of Langerhans occurred within a period of 6 to 8 days [19] The beta cells were degranulated to such an extent that in 3 dogs

The temporary diabetes elicited during the administration of anterior pituitary extracts was termed idiohypophyseal diabetes by Young[16] and the permanent pituitary diabetes persisting in dogs after the cessation of the anterior pituitary injections has been designated metahypophyseal diabetes by Houssay[17] In view of the multiple effects exerted by the impure extracts and as an aid to precision, these diabetic states elicited by purified growth hormone, may conveniently be referred to as idiosomatotropin diabetes (IS diabetes) and metasomatotropin diabetes (MS diabetes), respectively The word somatotropin is mentioned in an article by Evans[18] and somatotropin appears in the textbook of Selye[18a]

Temporary Pituitary Diabetes Produced by Growth Hormone An account of the characteristics of the temporary diabetes produced by the growth hormone would be incomplete without a description of the changes that take place prior to and which accompany, the hyperglycaemia and other changes that are usually considered as signs of diabetes The earlier deviations from the normal must be known if the mode of action of the hormone is to be understood The sequence of events which occurs when the growth hormone is given daily to dogs in doses adequate to produce diabetes i e 2 to 3 5 mg per kg of body weight, will therefore be mentioned From the beginning of the injections the body weight increases and the appetite appears to be keener The volume of the blood plasma per kg of body weight is increased by the growth hormone (Campbell Hausler Munroe and Davidson)[19] the increase being progressive during 5 days of injection[0] The volume of the red blood corpuscles per kg of body weight is not altered The blood volume is of course increased by the amount of the increase in the plasma volume The volume per cent of the packed red blood corpuscles is decreased due to the relative increase in plasma volume

The amount of total protein in the blood plasma is increased by the growth hormone prior to the appearance of diabetes The concentration of fibrinogen is raised up to three times the normal value and the amount of the protein in the circulating plasma is to an even greater extent increased due to the expansion of the plasma volume The concentrations of albumin and of serum globulin are not significantly increased by the administration of the growth hormone but the amounts of these proteins in the plasma are increased due to the increase in the plasma volume The growth hormone produces the greatest change in the fibrinogen but due to their relatively high concentrations the albumin and the serum globulin fractions contribute more to the increase in the total amount of protein in the plasma

The increase in the amount of any one of these protein components could be due, possibly, to an increase in their rate of entry into the plasma or to a decrease in the rate of removal or to both of these changes occurring simultaneously This question has been resolved by Webb Munroe and Campbell[0] who found by means of S^{35} labelled protein that growth hormone accelerated the rate of formation of fibrinogen without altering

blood are not dependent on the production of hyperglycaemia It is also clear that these effects can be present while a diabetic state exists

Permanent Diabetes Produced by the Growth Hormone Permanent diabetes produced by the growth hormone in dogs (metasomatotropin diabetes) has the same characteristics so far as they have been examined as the permanent diabetes produced by saline anterior pituitary extracts (Young [5]) and by partially purified anterior pituitary extracts (Marks and Young [6] Best Campbell and Haist [7]) This diabetes has been established in 8 dogs in this laboratory by average doses of 3 to 3 5 mg of growth hormone per kg of body weight per day for 15 to 37 days except for one instance mentioned below After these injections ceased the ketosis and lipaemia disappeared but the hyperglycaemia (200 to 400 mg per cent) the glycosuria (5 8 to 22 g per kg of body weight per day) and the polyuria remained Considering the extent of this drain from the body and the strains that must thereby be placed on the metabolic reserves and on the assimilative system this degree of diabetes is tolerated remarkably well These animals have been kept for as long as 150 days without insulin During this period there is however a gradual decline in body weight and in the condition of the animals Ketosis may reappear and anorexia and lethargy may become evident

On giving insulin a marked improvement in the vigor and appetite of the dogs occurs the body weight increases rapidly the ketosis vanishes and the blood and urine sugar levels can be closely controlled The insulin requirements in most instances are in the range of those that would be expected of the same dogs if depancreatized In one of these MS diabetic dogs however the insulin requirements increased gradually from about 18 to 54 units daily over a period of several months We are not aware of the causes of this increase In two dogs with MS diabetes the insulin requirements were not changed significantly by pancreatectomy

In some metahypophyseal diabetic dogs the insulin requirements were found to be higher than those usually found in depancreatized animals and pancreatectomy reduced the requirements (Marks and Young [6] Campbell Keenan and Best[29]) It has been suggested by Young [9] that this difference may be attributable to the removal of the alpha cells which may secrete the hyperglycaemic glycogenolytic factor (HGF) On removal of the pancreas from alloxan diabetic dogs the insulin requirements are also reduced and this effect has also been attributed to loss of the alpha cells (Thorogood and Zimmerman[30]) The recent studies of Grande and deOya [31] however reopen this issue

In the dogs with MS diabetes the acinar tissue of the pancreas has a normal appearance on histological examination The islets of Langerhans are however very few in number many sections can be examined without observing any structure resembling an islet The islets which are present appear to be of lesser size than in the normal These islets contain alpha

with temporary diabetes no stainable granules were found In one dog which resisted the diabetogenic effects of the growth hormone but in which the increases in the volume and the protein content of the plasma occurred granulation was found in the beta cells but much less than in the normal Thus even when diabetes is absent, as indicated by hyperglycaemia and glycosuria, degranulation of the beta cells of the pancreatic islets is produced by the growth hormone As was shown by Dr G A Wrenshall the insulin extractable from the pancreas is much reduced by the short period of treatment with growth hormone and the amount of insulin obtained is related to the degree of degranulation of the beta cells

In this state of IS diabetes in dogs the liver is much enlarged and the content of fat free solids per kg of body weight is increased to such an extent as to show an increase in liver protein The amount of total lipid in the liver is above normal and the degree of this fat deposition appears to be related to the reduction of the insulin extractable from the pancreas and to the severity of the diabetes The weight and the fat free solids of the kidney are increased in these dogs but these changes are not as pronounced as in the liver the fat content of the kidney is not definitely increased

As the administration of the growth hormone is continued the animals become obviously ill Lethargy weakness and anorexia are observed followed by vomiting and dehydration Prostration and death occurred in a dog after only 7 days of injection that produced severe diabetes These disturbances appear to be of the same nature as the toxic manifestations that were found by de Bodo Sinkoff Kiang and Den [3] in hypophysectomized dogs given lesser doses of growth hormone (1 mg per kg of body weight) In the hypophysectomized animals however it appears that hyperglycaemia and glycosuria did not accompany these toxic manifestations although the animals exhibited strong resistance to insulin and diabetic glucose tolerance curves These authors found that cortisone given daily was remarkably effective in reducing the toxic manifestations of the growth hormone in hypophysectomized dogs This discovery is probably of considerable importance although the mode of operation of the effect is not known as yet

It may be speculated that the changes in plasma volume and proteins might be caused by diabetes elicited by the growth hormone The plasma protein changes however precede the hyperglycaemia and in one instance were elicited without subsequent hyperglycaemia and glycosuria Not content with this evidence Munroe and Chaikoff[14] showed that when the caloric intake was reduced the same doses of growth hormone could produce the changes in plasma proteins but not the diabetes In the experiments of Webb Munroe and Campbell [20] the growth hormone accelerated the formation of certain plasma proteins but due to regulation of the dosage and diet diabetes was avoided It is therefore evident that the changes in plasma volume and proteins and also in the leucocytes of th

recovery lesions of the beta cells of the pancreatic islets persisted including slight degranulation and enlargement of the nuclei and in two instances hyaline degeneration and round-cell infiltration Young and Richardson [illegible] found that pituitary diabetic cats after many weeks of persisting diabetes could recover normal blood sugar levels but all the beta cells of the pancreatic islets that were seen in these animals exhibited hydropic degeneration Young[33] also observed a prediabetic state in a dog that had been injected during puppyhood and into the adult state with diabetogenic anterior pituitary extract The injections accelerated growth and after growth ceased, caused diabetes On ceasing the injections for an interval of 18 days the diabetes disappeared Then 4 daily injections of extract promptly elicited glycosuria and ketonuria indicating a prediabetic state It may be noted that in this dog the subsequent coincident administration of growth hormone and insulin caused resumption of the gain in body weight

In a dog studied in this laboratory a long course of injections of growth hormone had elicited diabetes which was not permanent The animal was nevertheless more sensitive to the diabetogenic effects of the growth hormone in that subsequent injections elicited hyperglycaemia and glycosuria more rapidly than in the normal state On ceasing the injections these signs of diabetes persisted longer before they returned to normal levels Between periods of injection the blood sugar was normal and no sugar was excreted One month after the last injection of growth hormone the dog was sacrificed and it was found that the pancreatic islets contained many degranulated beta cells and that the insulin extractable from the organ was subnormal in amount 0 42 units per g of pancreas i e about 1/10 of the normal In another animal which had been used on many occasions to test the diabetogenic activity of anterior pituitary extracts but in which the blood sugar was normal only 3 days of injection of the growth hormone were required to establish permanent diabetes whereas in other instances the shortest period of injection needed to induce the diabetes was 15 days

It would appear that in these cases the previous periods of injection of the growth hormone caused a permanent reduction in the insulin secreting capacity of the pancreas and a permanent lesion of the beta cells The remaining capacity appeared to be adequate for ordinary needs since a period of over feeding failed to produce diabetes in the first of these animals It may be considered however that these animals had a reduced resistance to diabetogenic factors and were in a prediabetic state

Mode of Action of the Growth Hormone A IMMEDIATE REDUCTION OF BLOOD SUGAR IN ABSENCE OF THE PANCREAS In acutely depancreatized dogs the immediate effect of the growth hormone is to cause a fall in blood sugar (Kurtz deBodo Kiang and Ancowitz[34]) In depancreatized dogs which had recovered from the operation maintained on insulin until 1 to 3 days prior to the experiment Sirek[35] found that growth hormone injection caused a

cells, some of which are of normal appearance while others appear to be atypic The beta cells if present, are not readily recognizable as they are degranulated and largely atrophic The proportion of the beta to the alpha cells is much less than in the normal islet Insulin can be obtained from these pancreases of dogs with MS diabetes but only in traces according to Dr G A Wrenshall

The fact that the permanently diabetic dogs can be kept in moderately good condition for months without insulin while excreting large amounts of sugar is of interest Under similar conditions, and while excreting not greater amounts of sugar depancreatized dogs would be expected to survive for several days only It is possible that in the permanently diabetic dog a minimal secretion of insulin from the residuum of beta cells permits of survival It is also possible that some other pancreatic factor missing in the depancreatized dog may be involved in the survival of the MS diabetic dog

Insulin appears to be the only special treatment that is required to maintain the permanently diabetic dogs in good condition indefinitely, up to 2½ years in dogs still living Pancreas is not required in the diet and the liver does not become fatty The lipotropic activity of the pancreas appears to be unaffected by the diabetes and the lesion of the islets of Langerhans In depancreatized dogs maintained on adequate amounts of insulin the liver becomes fatty unless raw pancreas or pancreatic extracts are provided It should also be noted that in MS diabetes the alpha cells though of normal appearance for the most part, are reduced in number This does not result however in any metabolic defect that has been recognized up to the present

It is evident from the above that the permanent diabetes produced in dogs by growth hormone can be attributed to lack of adequate secretion of insulin from the pancreas and that the defective supply of insulin can be related to the severe lesion of the beta cells of the islets of Langerhans

Partial Loss of Islet Function In earlier experiments [7] on temporary pituitary diabetes it was found that on ceasing the injections of growth hormone the hyperglycaemia and glycosuria disappeared in one or two days and that the insulin content of the pancreas was restored to normal levels somewhat less rapidly Intermediate stages between this apparently fully reversible type of temporary diabetes and permanent diabetes in dogs were not recognized but it now appears that intermediate prediabetic stages exist

Lukens and Dohan[3] found that partially depancreatized cats could recover from a metahypophyseal diabetic state induced by an anterior pituitary extract in that the hyperglycaemia and glycosuria disappeared The recovery was aided by undernutrition and by the administration of insulin and by phlorhizin However on repeated production of the diabetes the amount of extract required to re elicit the diabetes was reduced After

causes mobilization of lipid from tissues and its deposition in the liver (Greenbaum and McLean [41] White[4]) The deposition of lipid in the liver of the dog given growth hormone may be due therefore to a combination of insulin deficiency and a lipid mobilization from tissues produced by other means Considering the magnitude of the effect and the experimental condi tions it is probable that the effect would be due for the most part to insulin deficiency

The administration of increased amounts of insulin reduces the degrees of polyuria glycosuria and ketonuria in diabetic dogs given growth hormone Large doses of insulin 4 or 5 times the usual maintenance dose may be given without completely overcoming the diabetogenic effects of the growth hormone (Chaikoff and Campbell[43]) Such doses of insulin would almost surely produce convulsions and death if the growth hormone were not given

These diabetogenic extrapancreatic effects of the growth hormone can not be adequately accounted for by assuming inhibition of the action of insulin alone even if the inhibition were complete The effects of the growth hormone under these conditions were more fulminating and more rapidly lethal than was the withdrawal of the insulin injections in depancreatized dogs Indeed the results indicated that larger doses of growth hormone would produce an even more powerful effect

According to Houssay [17] in hypophysectomized depancreatized dogs the injection of saline extracts of anterior pituitary promptly exacerbates the diabetes and rapidly causes coma and death Milman DeMoor and Lukens[44] found that in hypophysectomized depancreatized cats growth hormone elicits glycosuria without influencing nitrogen excretion It is evi dent that these diabetogenic actions of the growth hormone in Houssay dogs and cats are exerted in the absence of insulin and other pancreatic factors

C EVIDENCE FOR STIMULATION OF INSULIN SECRETION BY THE GROWTH HORMONE Indirect evidence that the growth hormone may under certain conditions increase the rate of secretion of insulin from the pancreatic islets is obtained by comparing the effects of the hormone in intact animals with its effects either in animals without the pancreas or in animals with atrophic beta cells [35] The amounts of the growth hormone required to produce hyper glycaemia and glycosuria in intact dogs are about ten times as great as the amounts that increase sugar excretion in depancreatized and in MS-diabetic dogs given their usual doses of insulin In the normal animals also the sugar excretion appears after a longer interval and is of lesser degree The deposi tion of lipid in the livers of the intact animals is not as great and the changes in the kidney are less pronounced Evidently the presence of the pancreas and more specifically the functionally active beta cells of the islets of Langerhans greatly increases the resistance of the intact animals to the diabetogenic effects of the growth hormone If the growth hormone creates an increased demand for insulin in the extra pancreatic tissues of the normal

fall in blood sugar if the initial concentration of sugar in the blood did not exceed 200 mg per cent Above this initial level the growth hormone injection caused a rise in blood sugar and in all instances the level rose above the initial value within 4 hours from the time of injection In discussing the nature of this effect, the first mentioned authors considered the possibility that insulin may remain in the extrapancreatic tissues after operation and participate with the growth hormone in the reduction of blood sugar The stimulating effect of growth hormone on the uptake of glucose by the rat diaphragm appears to be dependent on insulin according to Ottaway,[36] who suggests that insulin either permits the tissue to respond to the growth hormone or that growth hormone may liberate insulin from an inactive complex deposited in the tissues According to Park, Brown Cornblath Daughaday and Krahl[37] growth hormone at first stimulates and later inhibits glucose uptake by the rat diaphragm These authors postulate that the hormone after its initial stimulating action is transformed in the tissue into an inhibitory factor However, alternative explanations for this effect are possible

B INCREASED DEMAND FOR INSULIN CREATED BY THE GROWTH HORMONE

Much experimental evidence indicates that the growth hormone creates an increased demand for insulin by the tissues of the body This evidence is partly derived from studies on the effects of the hormone in the dog from which the pancreas has been removed and in the MS diabetic dog in which the beta cells of the pancreatic islets have been rendered atrophic (Campbell Munroe Hausler and Davidson[38]) When these diabetic animals are given their usual maintenance doses of insulin injections of relatively small amounts of growth hormone promptly exacerbate the diabetes Within a day of the first injection the blood sugar is increased and lipaemia may appear The urine volume and the excretion of sugar and ketone bodies increase considerably After 4 or 5 days of injection of growth hormone with the usual doses of insulin the liver is greatly enlarged very fatty and friable The kidneys are also enlarged and fatty On histological examination in addition to numerous intracellular fat droplets glycogen infiltration is seen in the cells of the tubules Fatty degeneration of cardiac muscle has also been observed in these dogs These effects of growth hormone are apparently as great in the MS-diabetic dogs as in the depancreatized animals

Von Mering and Minkowski[39] observed that pancreatectomy in dogs caused in addition to other signs of diabetes a rapid and massive deposition of lipid in the liver Banting Best et al [40] demonstrated that the administration of pancreatic extracts containing insulin prevented this effect of pancreatectomy

It is reasonable to conclude that these diabetogenic effects of the growth hormone are due to a relative insufficiency of insulin caused by an increased demand or need for insulin by the extrapancreatic tissues

It will be recalled that in the fasting rat and mouse the growth hormone

and atrophy of hormone producing cells in the body (beta cells) by another hormone (growth hormone) appears to be without parallel in other hormonal relationships

The Control of the Secretion of Insulin Since the great demand for extra insulin created by the injection of growth hormone has little influence on the level of blood sugar for 2 to 4 days in the normal dog it is indicated that the amount of insulin secreted by the pancreas under these conditions can be increased far beyond the normal limits and that the rate of the secretion can be fairly closely controlled Studies from several laboratories demonstrate that the level of sugar in the blood influences the rate of secretion of insulin from the pancreas[49] When growth hormone is given to dogs this factor is apparently not adequate to explain the early changes in the beta cells and the reduction in the insulin extractable from the pancreas[19] It has been suggested by Ham and Haist[46] and by others that the secretion of insulin may be regulated by the concentration of insulin in the blood The recent studies of Bornstein, Reid and Young[56] Young[1] and Foa Magid Glassman and Weinstein[57] suggest that the growth hormone may stimulate the alpha cells of the islets of Langerhans to secrete glucagon and thereby stimulate the beta cells to secrete insulin

An important derivation from the study of diabetogenic activity is this evidence that when the growth hormone is introduced in excess parenterally into the dog and the cat it may operate together with increased amounts of insulin secreted from the pancreas Both hormones influence the metabolism of protein fat and carbohydrate but the primary processes which they modify are unknown The present evidence indicates that the action of these hormones may be focused on the processes of protein synthesis and fat oxidation Salter and Best[58] and Lawrence Salter and Best[9] found that protein formation can be stimulated by insulin in the absence of growth hormone In the absence of insulin, growth hormone does not promote nitrogen retention[1] It appears that a greater synthesis of protein is produced by the combined actions of these hormones than by the sum of their separate actions It is possible that, at the sites of action in the tissues the activities of these hormones in promoting protein synthesis may be linked at a reaction determining final rates of protein synthesis

Bartlett and Gaebler[60] found by the use of N^{15} labeled amino acids that the growth hormone increases the size of the nitrogen pool in dogs as the resultant of two over all effects i e a depressed rate of catabolism of amino acids and an increased rate of synthesis of protein Further studies of these investigators[61] indicate that the hormone increases the rate of formation of certain protein factions of the plasma As was mentioned above the growth hormone increases the rate of entry of fibrinogen and of albumin into the plasma Fibrinogen is produced by the liver and some of the albumin of the plasma may also be produced by the hepatic cells according to Madden and Whipple[6] Since the growth hormone causes coincident gains in liver

animal as it does in the diabetic animals, it follows that the resistance of the normal dog to the diabetogenic effects of the growth hormone may be attributed to increased activity of the beta cells of the islets of Langerhans in producing increased amounts of insulin The sensitivity of the MS-diabetic dog to the diabetogenic effects of the growth hormone can be attributed to the inability of the small residuum of beta cells to produce insulin at an increased rate The findings of Milman DeMoor and Lukens[1] that insulin is required with growth hormone for nitrogen retention in depancreatized and in Houssay cats also provide clear cut evidence though indirect that the growth hormone may call forth the secretion of insulin

Studies on the pancreatic islet cells also indicate that the growth hormone may increase the rate of production of insulin by the beta cells (Richardson and Young[45] Ham and Haist[46] Haist[47]) The granules of the beta cells appear to contain insulin in a stored inactive form Degranulation and glycogen infiltration (Duff and Toreson[48]) or hydropic degeneration of the beta cells can be produced by either the administration of large amounts of glucose or the partial pancreatectomy leading to diabetes in dogs (Brown et al[49] Allen[50]) These histological changes therefore appear to be associated with increased activity of the beta cells in the secretion of insulin Under these conditions the rate of secretion may be in excess of the rate of formation of insulin in the cell so that degranulation and the other changes occur The changes induced in the beta cells of the dogs given diabetogenic doses of growth hormone have the same histological features as those which occur in the beta cells of the remnant of pancreas after partial pancreatectomy (Haist Campbell and Best[51]) Fasting undernutrition and insulin are effective to varying extents in preventing these changes in the beta cells of partially depancreatized dogs and also of dogs given diabetogenic anterior pituitary extracts (Bowie[5] Best Campbell Haist and Ham) All these measures would be expected to reduce the strain on beta cells stimulated to insulin secreting activity beyond the usual limits That such measures rest the beta cells is indicated by the experiments of Haist and Best[54] In the normal rat fasting fat feeding and the administration of insulin reduce the amount of insulin extractable from the pancreas while the beta cells appear to be in a state of reduced activity According to Mirsky and colleagues prolonged administration of insulin to partially depancreatized dogs may so reduce the activity of the beta cells that diabetes may occur on ceasing the injections These studies on the cells of the islets of Langerhans provide evidence that the administration of growth hormone may stimulate the beta cells to increased activity in the secretion of insulin

These and other results lead to the postulate that the permanent diabetes produced in dogs by *diabetogenic anterior pituitary extracts* and by the growth hormone is caused by over stimulation of the beta cells of the islets of Langerhans which overwork is of so great an extent as to cause exhaustion and atrophy of these cells This demonstration of the over powering

Young[66] found that single injections of a non diabetogenic pituitary extract which was pancreotropic (Marks and Young[67 68]) alleviated the diabetes of pituitary diabetic dogs The effect disappeared as the severity of the diabetes increased but reappeared when the dogs were given some insulin

In the later experiments (Chaikoff and Campbell[13]) MS-diabetic dogs were maintained on their usual diet and insulin dosage and then were given small doses of growth hormone for about 5 days The glycosuria increased during these injections of growth hormone but the insulin requirements became much less than prior to the injections when the latter were stopped The reduction took place in about one week The insulin requirement then rose but remained less than the initial level for indefinite periods On repeating the injections of the growth hormone for 4 to 5 days in the same animals a subsequent reduction in the insulin requirement occurred but this reduction was of lesser extent than was obtained after the first treatment with growth hormone Repetitions of this treatment did not give further reduction of the insulin requirement Treatment of depancreatized dogs in the same way with growth hormone also caused reduction of the insulin requirements subsequent to the injections but the effect was not as definite as in the MS diabetic dogs

On biopsy of the pancreases of the MS-diabetic dogs during the phase of reduced insulin requirement no histological evidence of activity of the beta cells of the islets was observed It appears that the reduction in the insulin requirement following the administration of the growth hormone is not due to change in the production of insulin from the islets although this possibility should not be excluded The possibility is suggested that the treatments with growth hormone may cause a persistent decrease in the output of growth hormone from the pituitary glands of the animals In these experiments the glycosuria and hyperglycaemia are enhanced during the injections of growth hormone but are subsequently reduced through a persistent alteration of homeostatic levels

Finally I regret that it has not been possible to mention the many important contributions of other investigators I hope that the other papers in this symposium will repair some of these omissions My coworkers and I wish to thank Professor C H Best for his interest and support in our studies The investigations mentioned from this laboratory were aided by grants from the National Research Council and from the National Cancer Institute of Canada

References

1 Young F G *Recent Progr Hormone Research* **8** 471 (1953)
2 Li C H Evans H M and M E Simpson *J Biol Chem* **159** 353 (1945)
3 Ellis S Noda G Simpson M E and H M Evans *J Biol Chem* **209** 779 (1954)

weight and in fat free solids, while these proteins enter the plasma at an accelerated rate, the hormone must increase the rate of synthesis of these proteins by the cells of the liver

Reduction of Insulin Requirement Subsequent to Growth Hormone It has been mentioned that Lukens and Dohan[7] induced the recovery of partially depancreatized cats from persisting diabetes by underfeeding and by the use of insulin and phlorhizin and that Young[13] observed recovery from persisting pituitary diabetes in cats which were otherwise untreated or were given insulin Although the sugar levels were normal in these animals signs of islet lesions persisted these being very pronounced in the cats studied by Richardson and Young [63] No reports of recovery of dogs from metahypophyseal diabetes have appeared although the glycosuria of many animals in this condition has been controlled at low levels by the administration of insulin for long periods of time It is likely however that these dogs have not been given a proper chance to recover, having been kept too long untreated by insulin after the administration of the pituitary extract or growth hormone It may be that when a beta cell is driven to an atrophic state by the pituitary injections no treatment whatsoever may be able to bring it back to functional activity The signs of proliferative activity in islet cells noted by Richardson and Young[61] and by Ham and Haist[9] in dogs given pituitary extracts offers hope that some replacement of islet cells in the pancreas of the diabetic may be possible The actions of oestrogens and certain cortical steroids in preventing diabetes after partial pancreatectomy in the rat and in promoting islet cell proliferation have been described by Houssay and his colleagues[64] and by Houssay, Rodriguez and Cardeza[6] Such considerations prompted the following experiments While no evidence of regeneration of islet tissue in the diabetic dogs was obtained some reduction of the insulin requirement was found after the injections of the pituitary extracts and of growth hormone had ceased

Campbell Keenan and Best [8] found that the administration of a diabetogenic anterior pituitary extract to a metahypophyseal diabetic dog and to a depancreatized dog increased the insulin requirements from 20 and 26 units respectively to amounts in excess of 160 and 86 units On ceasing the injections of the extract the insulin requirements fell below the previous basal levels to 12 and 9 units respectively for 5 to 6 weeks

In another series of experiments (Haist Campbell and Best [1]) the insulin requirements of metahypophyseal diabetic dogs were determined The animals were then subjected to periods of fasting and given moderate doses of a diabetogenic globulin fraction of the anterior pituitary together with sufficient insulin which was injected frequently to keep the blood and urine sugar low After about 7 days of this regimen the pituitary injections were stopped the usual diet was restored and the insulin requirements were determined Some reduction of the insulin requirement was obtained by this treatment but the results obtained on repetition were not impressive

40 Banting F G Best C H Collip J B MacLeod J J R and M A Noble *Trans Roy Soc Can V* **13** 39 (1922)
41 Greenbaum A L and P McLean *Biochem J* **54** 407 (1953)
42 White A *Recent Progr Hormone Research* **4** 153 (1949)
43 Chaikoff L and J Campbell *Proc Can Physiol Soc Toronto* 1954 19
44 Milman A E DeMoor P and F D W Lukens *Am J Physiol* **166** 354 (1951)
45 Richardson K C and F G Young *Lancet* **1** 1098 (1938)
46 Ham A W and R E Haist *Am J Path* **17** 787 (1941)
47 Haist R E *Physiol Rev* **24** 409 (1944)
48 Duff L G and W E Toreson *Endocrinology* **48** 298 (1951)
49 Brown E M Jr Dohan F C Freedman L R deMoor P and F D W Lukens *Endocrinology* **50** 644 (1952)
50 Allen F M *J Metab Res* **1** 5 (1922)
51 Haist R E Campbell J and C H Best *New Eng J M* **223** 607 (1940)
52 Bowie D J M A Thesis University of Toronto 1925
53 Best C H Campbell J Haist R E and A W Ham *J Physiol* **101** 17 (1942)
54 Haist R E and C H Best *Science* **91** 410 (1940)
55 Mirsky I A Nelson N Elgart S and I Grayman *Science* **95** 583 (1942)
56 Bornstein J Reid E and F G Young *Nature* (London) **168** 908 (1951)
57 Foa P P Magid E B Glassman M D and H R Weinstein *Proc Soc Exp Biol Med* **83** 758 (1953)
58 Salter J M and C H Best *Brit M J* **2** 353 (1953)
59 Lawrence R T B Salter J M and C H Best *Brit M J* **2** 437 (1954)
60 Bartlett P D and O H Gaebler *J Biol Chem* **196** 1 (1952)
61 Bartlett P D and O H Gaebler *J Biol Chem* **196** 11 (1952)
62 Madden S C and G H Whipple *Physiol Rev* **20** 194 (1940)
63 Richardson K C and F G Young *J Physiol* **91** 352 (1937)
64 Houssay B A *Newer Concepts of the Causes and Treatment of Diabetes Mellitus* New York The National Vitamin Foundation Inc 1954 45
65 Houssay B A Rodriguez R R and A F Cardeza *Endocrinology* **54** 550 (1954)
66 Young F G *Brit M J* **2** 897 (1941)
67 Marks H M and F G Young *Lancet* **1** 493 (1940)
68 Marks H M and F G Young *Lancet* **2** 710 (1940)

4 Harris, J I Li C H Condliffe P and N G Pon *J Biol Chem* 209 133 (1954)
5 Reid E *J Endocrinology* 8 50 (1952)
6 Raben, M S and V W Westermeyer *Proc Soc Exp Biol Med* 80 83 (1952)
7 Reid E *J Endocrinology* 9 210 (1953)
8 Houssay B A and R R Rodriguez *Endocrinology* 53 114 (1953)
9 Campbell J and I W F Davidson *Proc IX Int Physiol Congress* Montreal
10 Campbell J Davidson J W F Snair W D and H P Lei *Endocrinology* 46 273 (1950)
11 Reid E *J Endocrinology* 9 185 (1953)
12 Milman A E DeMoor P and F D W Lukens *Am J Physiol* 166 354 (1951)
13 Reid E *J Endocrinology* 9 322 (1953)
14 Westermeyer V W and M S Raben *Endocrinology* 54 173 (1954)
15 Engel F L Viau A Coggins W and W S Lynn *Endocrinology* 50 100 (1952)
16 Young F G *Lancet* Dec 955 (1948)
17 Houssay B A *Endocrinology* 30 884 (1942)
18 Evans H M *J A M A* 104 464 (1935)
18a Selye H *Textbook of Endocrinology* Montreal Acta Inc 1947
19 Campbell J Hausler H A Monroe J S, and I W F Davidson *Endocrinology* 53 134 (1953)
20 Webb T Munroe J S and J Campbell (to be published)
21 Wintrobe M *Clinical Hematology* 11 ed Philadelphia Lea & Febiger 1946
22 deBodo R C Kurtz M Ancowitz A and S P Kiang *Am J Physiol* 163 310 (1950)
23 deBodo R C Sinkoff M W Kiang S P and H Den *Proc Soc Exp Biol Med* 81 425 (1952)
24 Munroe J S and L Chaikoff *Rev can biol* 13 77 (1954)
25 Young F G *Lancet* Aug 372 (1937)
26 Marks H M and F G Young *J Endocrinology* 1 470 (1939)
27 Best C H Campbell J and R E Haist *J Physiol* 97 200 (1939)
28 Campbell J Keenan H C and C H Best *Am J Physiol* 126 455 (1939)
29 Young F G *Acta Med Scand* 135 275 (1949)
30 Thorogood E and B Zimmerman *Endocrinology* 37 191 (1945)
31 Grande F and J C deOya *Bull Int Med Res Univ Madrid* 6 49 (1953)
32 Lukens F D W and F C Dohan *Endocrinology* 30 175 (1942)
33 Young F G *Brit M J* Dec 714 (1944)
34 Kurtz M deBodo R C Kiang S P and A Ancowitz *Proc Soc Exp Biol Med* 76 21 (1951)
35 Sirek O Ph D Thesis University of Toronto 1954
36 Ottaway J H *Biochim et Biophys Acta* 11 443 (1953)
37 Park R C Brown D H Cornblath M Daughaday W H and M E Krahl *J Biol Chem* 197 151 (1952)
38 Campbell J Munroe J S Hausler H A and I W F Davidson *Endocrinology* 53 549 (1953)
39 von Mering J and O Minkowski *Arch exp Pathol Pharmakol* 26 371 (1889)

glucagon preservation of muscle glycogen stores on fasting lowering of the blood sugar and promotion of milk secretion

It was of interest to inquire whether the doses of growth hormone preparations needed to induce these diverse effects differed greatly from those needed to cause growth It would be expected that if several effects are to be observed following the administration of a dose of a growth hormone preparation just large enough to elicit measurable growth the probability that they are due to growth hormone itself rather than to a contaminant or to a side action would be increased Thus the effects listed in Table 1 given

Table 1

EFFECTS ELICITED BY GROWTH HORMONE PREPARATIONS GIVEN IN DOSES OF LESS THAN 1 MG /KG

Effect	*Animal*		*Mg /Kg*	*References*
Widening of cartilage	H	Rat	0 01–0 3	1 2 3
Restoration of insulin response	H	Dog	0 02–0 3	4
Galactopoiesis	N	Cow	0 1	5
Growth	H	Rat	0 1–0 3	6 1
Hypoglycemia	H	Rat Dog	0 3–1 0	7 8

* N—Normal H—Hypophy ectomized

Table 2

EFFECTS OF GROWTH HORMONE PREPARATIONS REQUIRING DOSES IN EXCESS OF 1 MG /KG

Effect	*Animal*		*Dose Mg /Kg*	*References*
Diabetes	N*	Dog Cat	1 –3	9 10
Ketosis	N	Fasting rat	1 5–10	11 12 13
Glycostatic effect	H	Rat	1 –20	14 15
Fat mobilization	N	Fasting mouse	5 –250	16 17 18 19
Use of amino acids	N	Nephrectomized rat	10	20
Glycotropic effect	N	Rat	30	21

N—Normal H—Hypophysectomized

by doses comparable to those required to induce weight gain in hypophysectomized rats might be more probable actions of growth hormone than those shown in Table 2 which require much larger doses Objections to this line of reasoning are several and the listing of actions in order of increasing required dose fails to divide the several effects into clearly defined groups

A better approach would involve comparing the potency of various pituitary extracts Clearly a positive correlation between growth promoting activity and potency in one of the tests for another metabolic function

17

Relation of the Metabolic Effects of Corticotropin Concentrates to Growth Hormone

E B Astwood

Department of Medicine Tufts Medical School and New England Center Hospital Boston

It is often difficult to determine which effects elicited by the administration of a hormone constitute normal aspects of its physiologic function and which effects are side actions which are of no importance and play no part in the action of endogenously secreted hormone under normal circumstances Examples of such side actions are the aplastic anemia of does from large doses of estrogen the oxytocic and pressor effects of the antidiuretic hormone vasopressin and the follicle stimulating and luteinizing actions of human chorionic gonadotropin in the rat In the case of the anterior pituitary hormones it is particularly difficult to know which effects represent normal physiologic actions of a hormone and to know to which hormone to ascribe them Here the problem is greatly complicated by problems of purity of the several hormones and some apparent side actions may be ascribed to contamination of one hormone preparation by another hormone

A variety of metabolic effects have been shown to be given by preparations of growth hormone Some of these are consistent with growth while others seem to have little bearing upon processes of anabolism Positive balances of nitrogen phosphorus calcium sulfur sodium potassium and chloride widening and cellular changes in epiphyseal cartilage and increased incorporation therein of radiosulfur, enlargement and various chemical changes in the viscera and an increased use of depot fat when the diet is restricted are all effects which might be expected when anabolic processes are stimulated Effects of growth hormone preparations which seem to be unrelated to the promotion of growth include induction of diabetes inhibition of insulin ketogenesis reduced respiratory quotient secretion of

causing ketosis than growth hormone preparations They concluded that ketogenic potency did not correlate well with corticotropic activity and that ketosis was due to a separate factor contained in the extract

The rate of oxygen consumption in mice and in rats was increased by the corticotropin preparation and the respiratory quotient was decreased in fed animals and failed to rise in animals given glucose Large doses of growth hormone preparations did not cause these effects[30, 9]

Glycostatic, Glycotropic, and Hypoglycemic Effects

The loss of muscle glycogen upon fasting after hypophysectomy has been shown by Russell and others[14, 15] to be prevented by treatment with crude pituitary extracts and with growth hormone preparations In the normal rat muscle glycogen particularly that of the heart muscle has been shown by Adrouny and Russell[34] to increase progressively upon fasting Treatment with growth hormone preparations was found to accelerate greatly this increase It has been shown by Otto[35] that corticotropin also exerts a glycostatic effect in normal and in hypophysectomized rats In the normal fasting mouse corticotropin seems to be at least ten times as active as growth hormone preparations in increasing the glycogen in the heart[29] and in the fasting hypophysectomized rat another corticotropin preparation was found by Fønss Beck and Li[36] to be highly active in maintaining the glycogen stores of skeletal muscle

The anti insulin or glycotropic effects of pituitary extracts are difficult to compare because more than one type of action seems to be involved Chronic treatment with growth hormone type of extract may lead to hyperglycemia and eventually diabetes and may thus be regarded as anti insulin in one sense However similar extracts also intensify the diabetic state of depancreatized[37] or Houssay animals and restore to normal the increased sensitivity to insulin of the hypophysectomized animal[4]

In tests for their capacity to inhibit insulin acutely growth hormone preparations have usually been found weak or inactive the effect sometimes being noted only after some hours or after repeated injections Corticotropin concentrates on the other hand have been found to inhibit insulin in mice when given one hour before or concurrently with insulin The effect is independent of the adrenals[23]

Lowering of the concentration of glucose in the blood has been noted in mice rats and dogs following the injection of crude extracts growth preparations and corticotropin concentrates Hypophysectomized or adrenalectomized rats have been found to be more sensitive than normal animals[33, 21] while no such effects have been noted in diabetic rats or mice even though the diabetes was mild[1] On the other hand hypoglycemia still occurred following the injection of growth hormone preparations immediately after pancreatectomy in the dog[8] Insofar as comparisons are possible the most potent hypoglycemic preparation from the pituitary is purified corticotro

would favor the identity of the active principles involved and vice versa Some evidence has accumulated which indicates that various extracts of comparable growth promoting potency may not be equally diabetogenic glycostatic, or adipokinetic And, on the other hand a variety of pituitary extracts, some with little growth promoting activity have been found to induce one or more of these metabolic effects These extracts include the crude preparations and ultrafiltrates of Anselmino et al the heat stable specific metabolic principle of Collip and collaborators [3, 4] thyrotropic extracts [17] the very labile principle associated with some growth hormone preparations and, more recently, corticotropin concentrates made by the oxycellulose procedure [6, 7] Purified corticotropin has been shown to be free of growth promoting activity in the adrenalectomized hypophysectomized rat sustained by cortisone therapy and to be without diabetogenic activity in the intact dog However when tested in the mouse it is highly active in tests for glycotropic [9] glycostatic [9] and adipokinetic effects[10], it increases oxygen consumption reduces the respiratory quotient increases ketosis and causes a lowering of the blood sugar All of these effects can be obtained in the absence of the adrenals but some of them require cortisone for their exhibition Some but not all of these actions are to be observed also in other species, including the rat rabbit and dog but not thus far in man Most of the metabolic actions can be induced by corticotropin in doses which are much smaller than the required doses of growth hormone preparations

Mobilization of Fat, Ketogenesis, Ketosis and Lowering of the Respiratory Quotient

Using the method developed by Campbell [31, 32] Rosenberg[30] tested various pituitary extracts for their capacity to increase the concentration of fat in the liver of fasting mice Oxycel purified corticotropin in a dose range of 3 to 10 μg caused a proportional increase in response with increasing dose with larger doses up to 100 μg there was a gradual further increase By comparison crude corticotropin was about one thirtieth as active and was in turn 4 to 5 times as potent as whole anterior pituitary powder Thus about one third of the total adipokinetic activity of the pituitary tissue had found its way into the corticotropin fraction Growth hormone preparations were much weaker and seemed to differ in potency from one another The quantities of growth hormone preparations reported to be required to cause increases in liver fat in the mouse have varied from 0 1 to 10 mg and some have been found to be inactive Corticotropin concentrate seemed to act more quickly than growth hormone preparations and when the dose was divided and given every two hours greater responses were observed

Quantities of purified corticotropin comparable to those required to increase hepatic lipid also caused an increase in the ketone bodies of the blood and ketonuria in fasting mice In the normal or adrenalectomized rat Engle and Engle[33] found similar preparations to be much more active in

tical However, such a conclusion receives no support from physiologic observations if the pituitary partakes importantly in carbohydrate and fat metabolism by the secretion of a metabolic factor the factor would have to differ from corticotropin or else the metabolic changes would parallel adrenal cortical activation and there is no suggestion of such a parallelism

Summary and Conclusions

Some of the metabolic effects of crude pituitary extract including mobilization of depot fat ketosis depression of the respiratory quotient hypoglycemia resistance to insulin and preservation of glycogen stores in muscle formerly regarded as properties of growth hormone are brought about by the administration of purified preparations of corticotropin Although the factor responsible for these changes has not been separated from corticotropin there is no evidence that endogenously secreted corticotropin exerts any of these actions Growth hormone preparations seem to be associated with a factor which may impair the utilization of carbohydrate and lead to temporary and eventually permanent diabetes Chronic administration of such preparations thus leads to metabolic changes similar to those induced acutely by preparations of corticotropin

References

1 Greenspan F S Li C H Simpson M E and H M Evans *Endocrinology* 45 455 (1949)
2 Greenspan F S Li C H Simpson M E and H M Evans *Hormone Assay* Ed C W Emmens New York Academic Press Inc 1950 273
3 Campbell J Hausler H R Munroe J S and I W F Davidson *Endocrinology* 53 134 (1953)
4 deBodo R C and M W Sinkoff *Ann N Y Acad Sci* 57 23 (1953)
5 Cotes P M Crochton J A Folley S J and F G Young *Nature* 164 992 (1949)
6 Li C H Evans H M and M E Simpson *J Biol Chem* 159 353 (1945)
7 Park C R Brown D H Cornblath M Doughaday W H and M E Krahl *J Biol Chem* 197 151 (1952)
8 Kurtz M deBodo R C Kiang S P and A Ancowitz *Proc Soc Exp Biol Med* 76 21 (1951)
9 Campbell J Davidson I W F Snair W D and H P Lei *Endocrinology* 46 273 (1950)
10 Cotes P M Reid E and F G Young *Nature* 164 209 (1949)
11 Bennett L L Kreiss R E Li C H and H M Evans *Am J Physiol* 152 210 (1948)
12 Volk B W and S S Lazarus *Proc Soc Exp Biol Med* 83 151 (1953)
13 Lotspiech W D and V P Petersen *Am J Physiol* 176 232 (1954)
14 Russell J A and A E Wilhelmi *Endocrinology* 47 26 (1950)
15 Illingworth B A and J A Russell *Endocrinology* 48 423 (1951)
16 Weil R and S Ross *Endocrinology* 45 207 (1949)
17 Payne R W *Endocrinology* 45 305 (1949)
18 Levin L and R K Farber *Recent Progress in Hormone Research* New York Academic Press Inc 7 399 (1952)

pin [8,33] This same preparation, as well as several other types of extract including pituitrin causes hyperglycemia in the rabbit and in man [39]

The significance of these changes in blood sugar are difficult to assess In the mouse and rat where hypoglycemia seems related to the glycostatic and glycotropic effects it would appear that glycogenolysis and glucose utilization are both inhibited The concurrent mobilization of fat ketosis and lowered respiratory quotient implies that the extract effects conservation of carbohydrate at the expense of fat To what extent these changes will be found to occur in other species remains to be determined Similarly it is yet to be shown that the pituitary secretes a factor which exerts such effects under natural conditions

Preparations of Corticotropin

Most experiments on the metabolic effects of corticotropin concentrates have been made with material extracted with hot glacial acetic acid and purified with oxycellulose [6] Such preparations contain a major component which when isolated by chromatography on IRC 50 carboxylic acid resin by the method of Dixon et al [40] has been termed Corticotropin A by White [41] and when isolated by countercurrent distribution Corticotropin β by Bell [4] A similar peptide has been derived from sheep pituitaries by Li et al [43] and called Corticotropin α Upon digestion with pepsin eleven amino acids can be removed without loss of biological activity and it is believed [44] that the resulting peptide is that referred to by Brink et al [45] as Corticotropin B

Although these purified peptides have not been studied extensively each has been tested in mice and found to increase liver fat and to lower the concentration of blood glucose Within the limits of variation in these tests they do not differ significantly from one another or from the unfractionated oxycel concentrate

Further alterations in the major active component can be carried out without changing the corticotropic or metabolic potencies Treatment at room temperature for 30 minutes with 0 1 N sodium hydroxide converts this component into a less basic substance which travels more quickly on the IRC 50 column [9] a phenomenon noted also by Stack Dunne Dixon and Young [46] and to migrate more anodically upon electrophoresis on paper in alkaline buffers [47] Digestion with pepsin after the alkali treatment yields a basic substance which travels less rapidly to the cathode than the pepsin digest of the untreated material Finally alkali treatment after peptic digestion reduces the basicity of the product Thus alkali and pepsin can bring about changes which yield four products which differ in electrophoretic mobility on paper without causing significant alteration in either corticotropic or metabolic activity

Although of an entirely negative character this evidence suggests that corticotropin and the factor responsible for the metabolic changes are iden

18

Relationship of the Adrenal Cortex to the Diabetogenic Action of Growth Hormone*

R C de Bodo and N Altszuler
Department of Pharmacology New York University College of Medicine
New York N Y

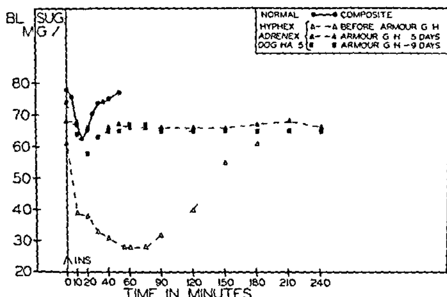
Studies carried out simultaneously in several laboratories during the past few years have definitely established that the pituitary growth hormone exerts a powerful effect on carbohydrate metabolism Several groups of investigators have shown that diabetes develops in normal[1 3] or partially depancreatized[4] dogs and cats after the continued administration of growth hormone Investigations carried out during this same period of time in our laboratory revealed that growth hormone ameliorates or abolishes the insulin hypersensitivity of hypophysectomized dogs and also produces diabetes in these animals[5 6 7]

In the present paper evidence is presented (1) that the diabetogenic action of the growth hormone preparations is due to growth hormone itself and not to the ACTH contaminant (2) that the adrenocortical steroids are not essential for the anti insulin and diabetogenic action of growth hormone (3) that the diabetes and toxic phenomena usually observed in hypophysectomized dogs on growth hormone regimens can be prevented or abolished by the administration of C 11 17 oxycorticosteroids and (4) that this beneficial effect of the steroids is probably related to an action on the insulin secreting function

---

The investigations reported here were supported by research grants A-113 (C2 C'S) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health Public Health Service by a grant in aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council and by a research grant from the Eli Lilly and Company

19 Szego C M and A White *Endocrinology* 44 150 (1949)
20 Russell J A and M Capiello *Endocrinology* 44 333 (1949)
21 Milman A E and J A Russell *Endocrinology* 47 114 (1950)
22 Hoffman F and K J Anselmino *Klin Wchschr* 12 1436 (1933)
23 O Donovan D K and J B Collip *Endocrinology* 23 718 (1938)
24 Derrick J B and J B Collip *Can J Research (E)* 31 117 (1953)
25 Greaves J D Freiberg I K and H E Johns *J Biol Chem* 133 243 (1940)
26 Astwood E B Raben M S Payne R W and A B Grady *J Am Chem Soc* 73 2969 (1951)
27 Astwood E B Raben M S Rosenberg I N and V W Westermeyer *Science* 118 567 (1953)
28 Westermeyer V W and M S Raben *Endocrinology* 54 173 (1954)
29 Astwood E B (unpublished observations)
30 Rosenberg I N *Proc Soc Exp Biol Med* 82 701 (1953)
31 Campbell J *Endocrinology* 23 692 (1938)
32 Best C H and J Campbell *J Physiol* 86 190 (1936)
33 Engel F L and M G Engel *Endocrinology* 55 834 (1954)
34 Adrouny G A and J A Russell *Federation Proc* 13 1 (1954)
35 Otto J F (unpublished observations)
36 Fonss Beck P and C H Li *J Clin Endocrinol and Metabolism* 14 834 (1954)
37 Campbell J Munroe J S Hausler H R and I W F Davidson *Endocrinology* 53 549 (1953)
38 Harrison H C and C N H Long *Endocrinology* 26 971 (1940)
39 Raben M S Personal communication
40 Dixon H B F Moore S Stack Dunne M P and F G Young *Nature* 168 1044 (1951)
41 White W F *J Am Chem Soc* 75 503 (1953)
42 Bell P J *Am Chem Soc* 76 5565 (1954)
43 Li C H Geschwind I I Levy A L Harris J I Dixon J S Pon N G and J O Porath *Nature* 173 251 (1954)
44 Hays E E and W F White *Recent Progr Hormone Research* 10 265 (1954)
45 Brink N G Boxer G E Jelinek V C Kuehl F A Jr Richter J W and K J Folkers *Am Chem Soc* 75 1960 (1953)
46 Stack Dunne M P Dixon H B F and F G Young Personal communication
47 Rosenberg I N (in press)



Fic 2 Blood sugar curves produced by insulin (0 025 U/kg i v) in normal dogs and in hypophysectomized adrenalectomized (Hyphex Adrenex) dog HA-5 during the untreated state and after 5 and 9 doses of growth hormone (G H) regimen (Armour lot #BGH 369-202B 1 mg /kg /day i m) (Courtesy of *Am J Phys ol* 176 362 (1954))

following the injection of insulin it gradually returned to the pre insulin level

In contrast to these exaggerated responses to insulin seen in the adrenal ectomized hypophysectomized dogs normal dogs showed only a slight fall in their blood sugar after such a small dose of insulin and the blood sugar returned to the postabsorptive level within 40 minutes (Figs 1 and 2)

The administration of growth hormone 1 mg /kg /day produced a marked anti insulin effect in the adrenalectomized hypophysectomized dogs HA 7 and HA 5 (Figs 1 and 2) i e the fall of the blood sugar following the injection of insulin 0 025 U/kg i v was greatly diminished as compared with the blood sugar changes seen in the untreated state and the recovery of the blood sugar to postabsorptive levels was markedly accelerated

The effect of growth hormone on the response to intravenous glucose of adrenalectomized hypophysectomized dogs is illustrated in Figure 3 It can be seen that the adrenalectomized hypophysectomized dog HA 7 prior to growth hormone therapy showed a very severe secondary hypoglycemia in the intravenous glucose tolerance test Thus following the intravenous infusion of glucose (0 075 g /kg /min for 10 minutes) the blood sugar fell rapidly from the peak value it had reached at the termination of the infusion to levels far below the postabsorptive level In dog HA 7 this second

Effects of Growth Hormone on the Responses to Insulin and Intravenous Glucose of Adrenalectomized Hypophysectomized Dogs

In order to decide whether the anti insulin and diabetogenic actions obtained with growth hormone preparations are due to growth hormone itself or to the ACTH present in these preparations the effects of these preparations were studied in adrenalectomized hypophysectomized dogs[8 9 10 11] If the effects observed in hypophysectomized animals were due to the ACTH contaminant of growth hormone, then no action should occur in the adrenalectomized hypophysectomized animal This is to be expected since it was shown that the carbohydrate metabolism of adrenalectomized dogs was not affected by ACTH i e, no extra adrenal effect of ACTH on carbohydrate metabolism could be demonstrated

In Figures 1 and 2 are shown the effects of growth hormone on the insulin response of adrenalectomized hypophysectomized dogs HA 7 and HA 5 maintained on minimal daily doses of DCA (1 5–2 5 mg) These dogs in the untreated state (prior to growth hormone therapy) showed precipitous falls in blood sugar following the intravenous injections of the "test dose" of insulin (0 025 U/kg) Since in dog HA 7 insulin caused severe hypoglycemic crises the experiments had to be terminated by treatment with sugar infusions in dog HA 5 although the blood sugar fell to very low levels

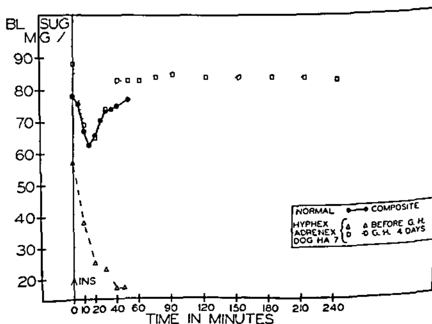


FIG 1 Blood sugar curves produced by insulin (0 025 U/kg i v) in normal dogs and in hypophysectomized adrenalectomized (Hyphex Adrenex) dog HA-7 during the untreated state and after 4 doses of growth hormone (G H) regimen (Armour lot #J21609R 1 mg /kg /day i m)

ary hypoglycemia was so profound that the animal developed convulsions In contrast to this in normal dogs following the intravenous glucose infusion the blood sugar fell from the peak value to the postabsorptive level and then stabilized at this level without falling below it

The growth hormone regimens reversed the abnormalities of the glucose tolerances of adrenalectomized hypophysectomized dogs The secondary hypoglycemia was abolished so that the glucose tolerance resembled more closely those of normal dogs (Fig 3)

Comment Since the administration of growth hormone to adrenalectomized hypophysectomized dogs maintained on small doses of DCA, (1) abolished their severe insulin hypersensitivity and (2) concomitantly abolished the secondary hypoglycemia of their intravenous glucose tolerance tests and (3) since ACTH has no extra adrenal effect on carbohydrate metabolism it is concluded that the action of growth hormone on carbohydrate metabolism is not due to the ACTH contaminant of the growth hormone preparation

Since in the above experiments on adrenalectomized hypophysectomized dogs growth hormone exerted its action in the absence of the adrenocortical steroids it must be further concluded that the adrenocortical steroids are not essential for the actions of growth hormone on carbohydrate metabolism

Replacement Therapy with Growth Hormone Complicated by (a) Diabetes (b) Toxicity and (c) Acquired Resistance

Having established that the observed effects on carbohydrate metabolism were indeed due to growth hormone and not to its ACTH contaminant the question arose whether these actions of growth hormone are of physiological significance If growth hormone has any physiological role in carbohydrate metabolism then the production of diabetes in normal or partially depancreatized animals by this hormone must be regarded as a pathological state produced by an excess of growth hormone which at least in its end effect is antagonistic to the action of insulin It appeared that valuable information might be obtained if the effects of growth hormone were studied in hypophysectomized animals It seems reasonable to assume that some of the abnormalities in carbohydrate metabolism manifested by hypophysectomized animals are due to the lack of growth hormone and should be ameliorated or restored by this hormone Thus it might be postulated that if growth hormone has a physiological role in carbohydrate metabolism and is an insulin antagonist then in proper dosage regimens it should be able to ameliorate or abolish the insulin hypersensitivity without showing any untoward effects

Indeed in a large series of hypophysectomized dogs an amelioration or abolition of the insulin hypersensitivity could be readily demonstrated with varying dosage regimens of growth hormone However several difficulties

Fig 3 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in normal dogs and in hypophysectomized adrenalectomized (Hyphex Adrenex) dog HA-7 during the untreated state and after 7 doses of growth hormone (G H) regimen (Armour lot #J21609R 1 mg /kg /day i m)

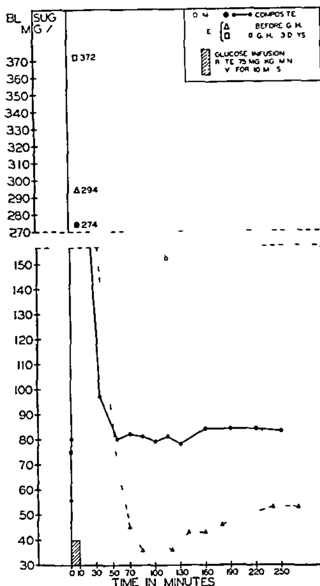


FIG 5 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in normal dogs and in a typical hypophysectomized (Hyphex) dog during the un treated state and after 3 doses of growth hormone (G H) regimen (Armour lot #22KR1 1 mg /kg /day i m) (Courtesy of *Diabetes* 3 89 (1954))

were encountered in these experiments in which replacement therapy with growth hormone was the objective

If growth hormone (about 1 mg /kg /day) was administered to hypophysectomized dogs which had manifested an insulin hypersensitivity and a marked secondary hypoglycemia following the intravenous glucose infusion their exaggerated insulin response was restored to normal within 3–4 days[6,7] (Fig 4) however their glucose tolerance instead of becoming normal changed to a diabetic type (Fig 5) Only very rarely was it possible to observe a transient normal glucose tolerance lasting for a few days (Fig 6) before it became diabetic

The growth hormone regimen also produced toxic phenomena in these animals such as anorexia complete refusal of food vomiting of force fed food weakness lethargy and death[7,8,9] When the toxic effects became severe the growth hormone administration had to be discontinued in order to save the animal When the toxic effects were not too severe and the animals survived the continued administration of growth hormone then after about 3–4 weeks the anti insulin and diabetogenic effects began to diminish and eventually disappeared completely[1]

The gradual development of a resistance to growth hormone when the latter was administered daily for several weeks occurred regularly This

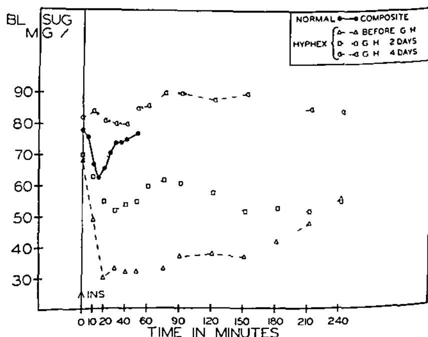


Fig 4 Blood sugar curves produced by insulin (0 025 U/kg i v) in normal dogs and in a typical hypophysectomized (Hyphex) dog during the untreated state and after 2 and 4 doses of growth hormone (G H) regimen (Armour lot #22KR1 1 mg /kg /day i m) (Courtesy of *Diabetes* 3 89 (1954))

acquired resistance to growth hormone proved to be specific for the species from which the hormone was obtained and is illustrated in Figures 7 and 8 When beef growth hormone was first administered to dog K7 3 for 7 days the secondary hypoglycemia of the glucose tolerance test was abolished On the 19th day of continued beef growth hormone regimen the secondary hypoglycemia reappeared signifying a diminished hormonal effect (Fig 7) Growth hormone administration was then discontinued for 11 days, and

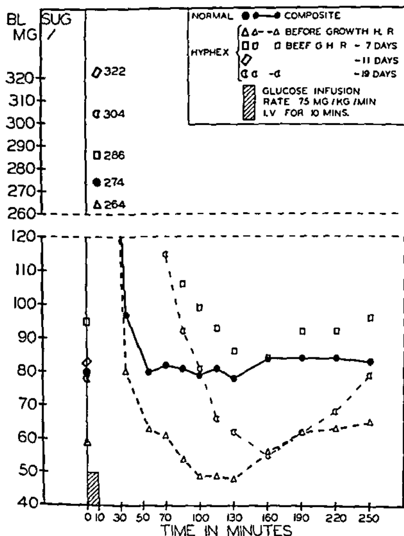


FIG 7 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in normal dogs and in hypophysectomized (Hyphex) dog K7-3 during the un treated state and after 7 11 and 19 doses of growth hormone (G H) regimen (Armour Lot #GH 3 1 mg /kg /day i m) Note the development of resistance

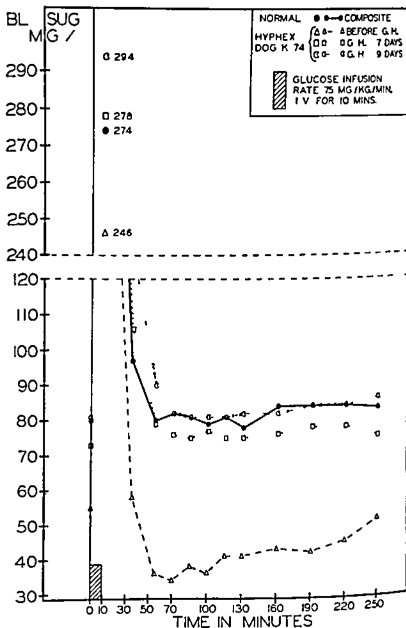


FIG 6 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in normal dogs and in hypophysectomized (Hyphex) dog K–74 during the untreated state and after 7 and 9 doses of growth hormone (G H) regimen (Squibb lot #C515 1 mg /kg /day i m)

when dog K7 3 was subsequently placed on a second beef growth hormone regimen it was ineffective On the other hand, when porcine growth hormone was administered it exerted an action (Fig 8)

Comment From the foregoing it is apparent that replacement therapy with growth hormone could not be readily achieved When the dosage of growth hormone was relatively large the development of diabetes and toxicity necessitated termination of the experiment If however the dose of growth hormone was greatly reduced (so that it did not even abolish the insulin hypersensitivity but only ameliorated it) its administration became feasible without toxicity but the acquired resistance made the experiment futile

Effects of Cortisone or Hydrocortisone on the Carbohydrate Metabolism of Hypophysectomized Dogs (a) in the Untreated State and (b) during Growth Hormone Regimen

Studies concerned with the hormonal regulation of renal functions revealed that the hypophysectomized dog had greatly reduced glomerular filtration rate and renal plasma flow and was also unable to excrete an orally administered water load [13 14] On either a growth hormone[1 16] or a C 11 17 oxycorticosteroid hormone[1 18] regimen the renal functions were only partially restored toward normal However on a combined steroid growth hormone regimen the glomerular filtration rate and renal plasma flow were restored to normal levels and the response to a water load became practically normal [8 9] Since these animals did not have diabetes and did not manifest toxic phenomena [19 8 9] it seemed worthwhile to explore the effects of a combined adrenocortical steroid growth hormone regimen upon carbohydrate metabolism

Experiments with Insulin First to be considered are the effects of these hormones and hormone combinations upon the responses to insulin in hypophysectomized dogs It can be seen in Figure 9 (dog K9 8) that on a cortisone regimen (1 0–1 4 mg /kg /day for 8 and 17 days) the insulin hypersensitivity of the hypophysectomized dog was ameliorated but not abolished i e the postabsorptive blood sugar was somewhat elevated the fall of blood sugar was still far greater than in normal animals and the return of blood sugar to the pre insulin level still considerably retarded The dosage of cortisone used in these experiments had been found previously to be sufficient to abolish the abnormalities of the carbohydrate metabolism of adrenalectomized dogs [0 8] On a subsequent combined cortisone growth hormone regimen the postabsorptive blood sugar level was further elevated and the response to insulin was about normal (Fig 9)

Experiments with Intravenous Glucose Before discussing the effects of the C 11 17 oxycorticosteroids and the combined steroid growth hormone regimens upon the glucose tolerance of hypophysectomized dogs the intravenous glucose tolerance curves of untreated hypophysectomized dogs

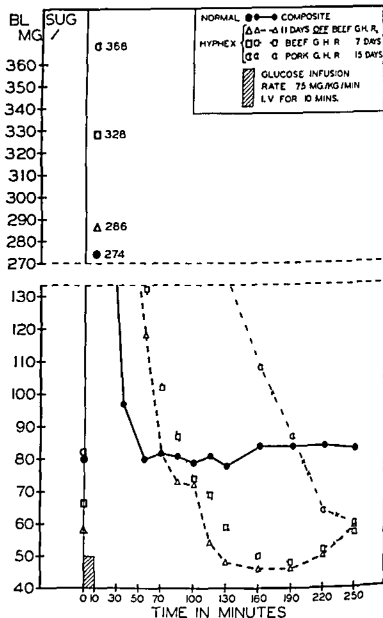


FIG 8 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in (a) normal dogs and in hypophysectomized (Hyphex) dog K7-3 (b) 11 days off beef growth hormone regimen (c) after 7 doses of a second beef growth hormone regimen (Armour lot #GH 3 1 mg /kg /day i m) and (d) after 15 doses of porcine growth hormone regimen (Armour lot #R491137 1 7 mg /kg /day i m) This porcine growth hormone regimen was started immediately after the second beef growth hormone regimen was discontinued

that the ability to secrete insulin may determine the type of glucose tolerance observed the underlying factors responsible for the production of the two types of glucose tolerances are still unknown

When a hypophysectomized dog manifesting the Type I glucose tolerance was placed on a cortisone or hydrocortisone regimen (1 0–1 4 mg /kg / day) (1) the postabsorptive blood sugar was usually somewhat elevated

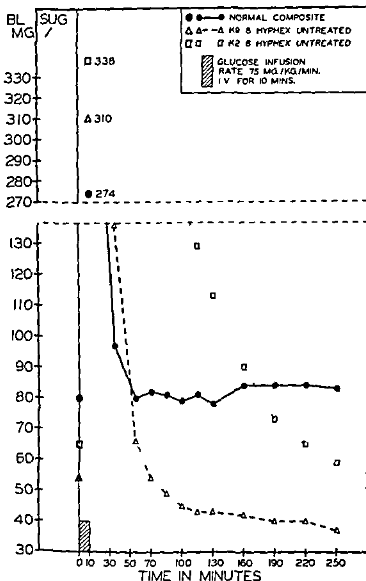


FIG 10 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in normal dogs and in hypophysectomized (Hyphex) dogs K9–8 and K–28 during the untreated state

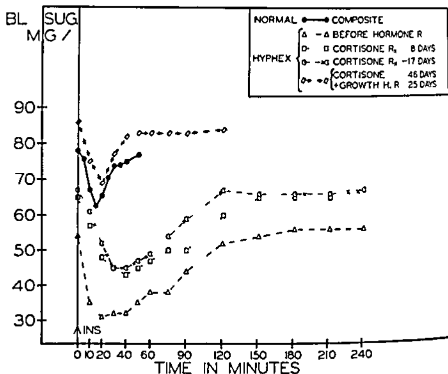


FIG 9 Blood sugar curves produced by insulin (0 025 U/kg i v) in (a) normal dogs and in hypophysectomized (Hyphex) dog K9–8 (b) during the untreated state (c) during cortisone regimen alone (after 8 and 17 doses) and (d) during a combined cortisone growth hormone regimen Cortisone (1 0–1 4 mg /kg /day) given intramuscularly for a total of 46 days growth hormone regimen (Squibb lot #C515 1 0–1 5 mg /kg /day i m) begun on the 22nd day of cortisone regimen and continued concomitantly for 25 days (Courtesy of *Diabetes* 3 89 (1954))

should be analyzed A study carried out on a large number of dogs revealed the existence of two types of glucose tolerance curves in the first type (Type I) the blood sugar fell rapidly from the peak value (obtained at the end of the glucose infusion) as in normal animals but instead of stabilizing at the pre infusion level it fell below it (secondary hypoglycemia) (Fig 10 dog K9 8) In the second type (Type II) the fall in the blood sugar from the peak value was very slow (almost as in a diabetic animal) but eventually after a few hours the secondary hypoglycemia still appeared (Fig 10 dog K 28) At present no experimental evidence is available to satisfactorily explain the variations in the glucose tolerance curves of the hypophysectomized dogs However an analysis of the glucose tolerance curves of hypophysectomized depancreatized animals wherein the blood sugar did not fall for as long as 5 hours [1] suggests that in the hypophysectomized dogs manifesting the Type II glucose tolerance the slow fall in the blood sugar was probably due to an impaired insulin secretion While it may be surmised

(2) the blood sugar still fell from the peak value as rapidly as in the untreated state and (3) the secondary hypoglycemia was diminished (dog K9 8 Fig 11 and dog F 3 Fig 12) When hypophysectomized dogs manifesting the Type II glucose tolerance were placed on a cortisone or hydrocortisone regimen (1) the postabsorptive blood sugar was somewhat

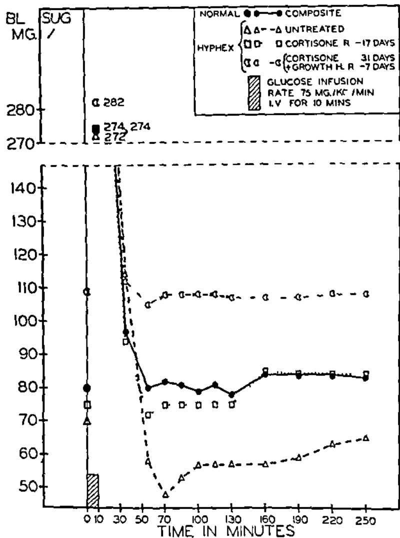


Fig 12 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in (a) normal dogs and in hypophysectomized (Hyphex) dog F–3 (b) during the untreated state (c) during cortisone regimen alone and (d) during a combined cortisone growth hormone regimen Cortisone (4 0–8 0 mg /kg /day) given intramuscularly for a total of 31 days Growth hormone regimen (Squibb lot #C515 1 5 mg /kg /day i m) was begun on the 25th day of cortisone regimen and continued concomitantly for 7 days

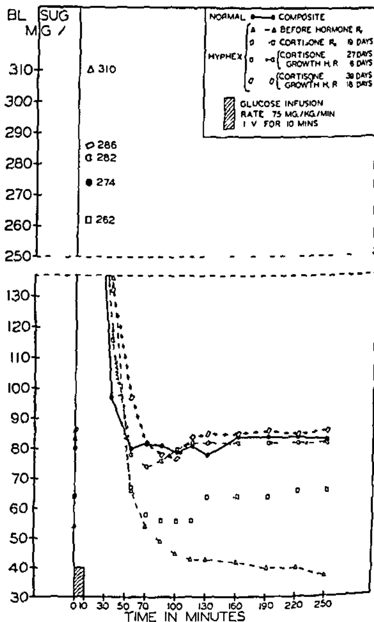


FIG 11 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in (a) normal dogs and in hypophysectomized (Hyphex) dog K9-8 (b) during the untreated state (c) during cortisone regimen alone and (d) during a combined cortisone growth hormone regimen Cortisone (1 0–1 4 mg/kg /day) given intramuscularly for a total of 39 days Growth hormone regimen (Squibb lot #C515 1 mg /kg /day i m) begun on the 22nd day of cortisone regimen and continued concomitantly for 18 days (Courtesy of *Diabetes* 3 89 (1954))

1 The postabsorptive blood sugar was always considerably elevated either to normal level as in dog K9 8 (Fig 11) and dog K 44 (Fig 13) or to levels far above normal as in dog F 3 (Fig 12) and dog K-28 (Fig 14)

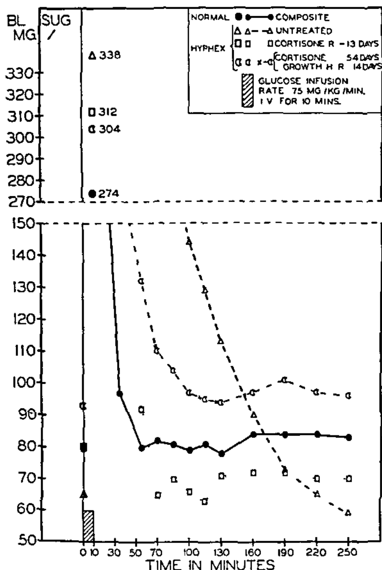


FIG 14 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in (a) normal dogs and in hypophysectomized (Hyphex) dog K-28 (b) during the untreated state (c) during cortisone regimen alone and (d) during a combined cortisone growth hormone regimen Cortisone (1 mg /kg /day i m) given for a total of 54 days Growth hormone (Armour lot #J21609R 1 mg /kg /day i m) begun on the 40th day of cortisone regimen and continued concomitantly for 14 days

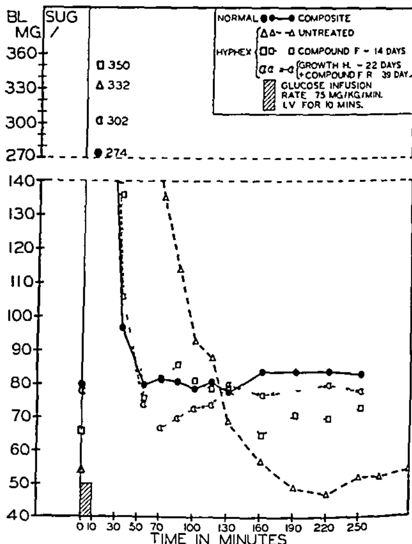


FIG 13 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in (a) normal dogs and in hypophysectomized (Hyphex) dog K–44 (b) during the untreated state (c) during hydrocortisone regimen and (d) during a combined hydrocortisone growth hormone regimen Hydrocortisone (0 83 mg/kg /day i m) given for a total of 39 days Growth hormone regimen (Armour lot #J21609R 1 0–1 5 mg /kg /day i m) was begun on the 18th day of hydrocortisone regimen and continued concomitantly for 22 days

elevated (2) the fall in blood sugar from the peak value was greatly accelerated resembling that of Type I dogs and (3) the secondary hypoglycemia was decreased (dog K-44 Fig 13 and dog K-28 Fig 14)

Substitution of the cortisone (or hydrocortisone) regimen by a combined cortisone (or hydrocortisone) growth hormone regimen produced the following changes in the intravenous glucose tolerance curves of hypophysectomized dogs

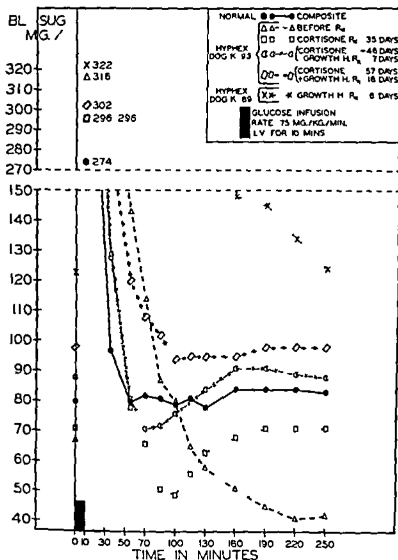


Fig 15 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in normal dogs and in hypophysectomized (Hyphex) dogs K–89 and K–93 Dog K–89 was given growth hormone (Squibb lot #C515 2 mg kg day i m) for 6 days Glucose tolerance curves of dog K–93 (a) in the untreated state (b) during cortisone regimen alone and (c) during combined cortisone growth hormone regimen Cortisone (1 0–2 0 mg /kg /day i m) given for a total of 57 days Growth hormone (Squibb lot #C515 1 mg /kg day i m) was begun on the 40th day of cortisone regimen and continued concomitantly for 7 days Then the dosage was increased to 2 mg /kg /day and continued concomitantly for 11 days

2 The blood sugar fall from the peak values remained rapid in both types of hypophysectomized dogs [Type I K9 8 (Fig 11) F 3 (Fig 12), Type II K 44 (Fig 13) and K 28 (Fig 14)] just as before growth hormone administration

3 The blood sugar falling from the peak value stabilized at the pre infusion level i e either at normal levels as in dog K9 8 (Fig 11) and dog K 44 (Fig 13) or at levels higher than normal as in dog F 3 (Fig 12) and dog K 28 (Fig 14) Thus the secondary hypoglycemia was completely abolished

The level to which the postabsorptive blood sugar may rise during a combined steroid growth hormone regimen appears to be determined by several factors In this respect the dose of growth hormone the duration of growth hormone administration and the degree of secondary hypoglycemia in the glucose tolerance test seem to be important When the dose of growth hormone in the combined hormone regimen was about 1 0 mg/kg /day the postabsorptive blood sugar reached the normal level and usually did not go higher (dogs K9 8 Fig 11 and K 44 Fig 13) Occasionally however even on this dosage regimen the postabsorptive blood sugar was above the normal level (dog K 28, Fig 14) When the dose of growth hormone in the combined hormone regimen was increased to 1 5–2 0 mg /kg /day the postabsorptive blood sugar was usually far above normal (dog F 3 Fig 12 and dog K 93 Fig 15) A gradual elevation of the postabsorptive blood sugar with the increase of growth hormone dosage (from 1 0 mg /kg /day to 2 0 mg /kg /day) may be noted in dog K 93 Fig 15 If the secondary hypoglycemia of the glucose tolerance test was not very marked the postabsorptive blood sugar usually reached a higher level on the combined hormone regimen

These glucose tolerance curves observed in hypophysectomized dogs during combined cortisone (hydrocortisone) growth hormone regimens were in sharp contrast to those obtained when growth hormone alone was administered While in the former the blood sugar dropped rapidly and then stabilized at normal or somewhat higher levels in the latter the fall was greatly retarded The severe diabetes produced by only three doses of growth hormone (1 mg /kg /day) in hypophysectomized dog K 8 Figure 5 and by six doses of growth hormone (2 0 mg /kg /day) in hypophysectomized dog K 89 Figure 15 should be noted In all the experiments in which the effects of combined steroid growth hormone regimens were studied great care was taken to evaluate the effect of this therapy long before resistance to the growth hormone could have developed Thus it is apparent that during the combined hormone regimens the steroids exerted their beneficial actions while the animals still reacted to growth hormone It is evident then that the administration of cortisone or hydrocortisone prior to and also simultaneously with growth hormone prevented the development of diabetes

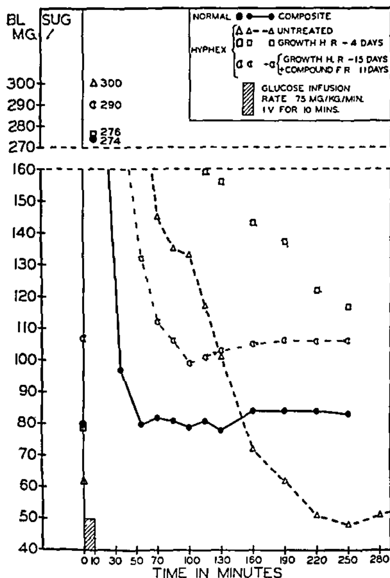


FIG 16 Blood sugar curves produced by intravenous glucose (0 75 g /kg) in (a) normal dogs and in hypophysectomized (Hyphex) dog Al-9 (b) during the untreated state (c) during growth hormone regimen (first) and (d) during a combined growth hormone hydrocortisone regimen Growth hormone (Armour lot #K90407R 1 mg /kg /day i m) given for 4 days hydrocortisone regimen (1 2 mg /kg /day i m) begun on the 5th day of growth hormone regimen and given concomitantly for 11 days

In addition, the steroids also prevented the development of the toxic phenomena usually produced by growth hormone The toxic manifestations as described above, were most probably not related to the diabetogenic action of the hormone Dogs which died from growth hormone toxicity did not show any evidence of either acetonemia or acidosis In fact, the hypophysectomized dogs exhibiting a diabetic type of glucose tolerance due to growth hormone did not reveal any glycosuria This may have been due to the fact that the animals perished long before the diabetes became severe enough to cause either glycosuria or acetonuria The mechanism of the growth hormone toxicity seen in hypophysectomized dogs is as yet not clear

In all the experiments discussed thus far in which combined steroid growth hormone regimens were used the cortisone or hydrocortisone was administered for varying periods of time prior to the steroid growth hormone regimen and under these conditions the steroids prevented the development of diabetes and toxicity It seemed important to investigate whether the steroids could also abolish or ameliorate the diabetes and toxicity already produced by growth hormone i e to administer growth hormone first and when diabetes developed to change the hormone regimen to a combined cortisone (hydrocortisone) growth hormone regimen In such an experiment it would be important to administer growth hormone alone for a relatively short period yet long enough to produce a well defined diabetes The initial hormone regimen should then be changed to a combined steroid growth hormone regimen administered for a time period adequate for the steroids to exert their beneficial effects but still not sufficiently long to permit development of a resistance to the action of growth hormone In Figure 16 it can be seen that hypophysectomized dog A1 9 was given growth hormone 1 mg /kg /day (without steroids) for 4 days as a result of which diabetes and toxicity developed Then a combined hydrocortisone growth hormone regimen was started and after 11 days the diabetes was ameliorated as judged by the rapid fall of the blood sugar from the peak value following the intravenous infusion of glucose However the blood sugar stabilized at a level higher than normal The toxic phenomena also disappeared Thus it is evident that the steroids not only can prevent the development of diabetes and toxicity of subsequently administered growth hormone but can also ameliorate or abolish these untoward effects already produced by growth hormone

Comment An analysis of the experiments in which cortisone (or hydrocortisone) was administered to hypophysectomized dogs reveals that the steroids produced the following changes (1) they raised the post absorptive blood sugar (2) they antagonized the action of administered insulin (3) they diminished the severity of the secondary hypoglycemia of the intravenous glucose tolerance tests and (4) they accelerated the fall of the blood sugar in these tests The well established action of the C 11 17 oxycorticosteroids namely that they enhance gluconeogenesis [23], and their

Finally consideration should be given to the growth hormone adreno cortical steroid relationship The C 11 17 oxycorticosteroids not only can prevent the development of growth hormone diabetes and toxicity but they can abolish or ameliorate these untoward effects produced by prior growth hormone treatment Although it appears that in the adrenalectomized dog the endogenous growth hormone exerts an anti insulin action in the absence of the adrenocortical steroids without causing diabetes or toxicity the im portance of the steroid growth hormone relationship cannot be denied The experiments presented in this paper clearly reveal an important action of the adrenocortical steroids Following the intravenous infusion of glucose into hypophysectomized dogs these steroids accelerate the removal of the extra sugar from the bloodstream perhaps by increasing the secretion or release of insulin When given with growth hormone the steroids still exert this action and thereby prevent or abolish the diabetes usually produced by growth hormone

It appears then that there exists a well adjusted balance between growth hormone insulin and the adrenocortical steroids in the regulation of carbo hydrate metabolism The following working hypothesis is suggested Both growth hormone and the adrenocortical steroids are blood sugar raising agents the former by inhibiting the peripheral utilization of sugar and the latter by increasing gluconeogenesis and perhaps also by inhibiting sugar utilization In this respect growth hormone and the adrenocortical steroids are synergistic and can be regarded as insulin antagonists But at the same time the adrenocortical steroids enhance the ability to secrete insulin which in turn antagonizes the action of growth hormone as regards sugar utiliza tion The interplay of these hormones in the normal organism is so perfect that although both diabetogenic substances namely growth hormone and the adrenocortical steroids are exerting their actions the animal still does not manifest diabetes

Summary and Conclusions

1 Administration of growth hormone ameliorated or abolished the severe insulin hypersensitivity of adrenalectomized hypophysectomized dogs maintained on minimal doses of DCA

2 Concurrently growth hormone decreased the severity of the second ary hypoglycemia of the glucose tolerance tests

Thus it may be concluded that the action of growth hormone on carbo hydrate metabolism is not due to its ACTH contaminant and that the adrenocorticosteroids are not essential for this action

3 Continued administration of growth hormone to hypophysectomized dogs in dosages sufficient to abolish or ameliorate their insulin hypersensi tivity produced diabetes toxic phenomena or resulted in a development of species specific resistance to growth hormone With small doses of growth

still disputed action namely that they interfere with the peripheral utilization of sugar[4, 6, 7, 8, 130] could explain the changes enumerated above under 1 2 and 3 However these actions could not explain the acceleration of the fall of blood sugar in the glucose tolerance tests It must be assumed that this is an additional action of the C 11 17 oxycorticosteroids hitherto not described This action of the steroids namely that they accelerate the fall of blood sugar in hypophysectomized dogs following intravenous glucose administration also seems to be responsible for the beneficial effects of the steroids in preventing or abolishing the growth hormone induced diabetes It is not unlikely that the adrenocortical steroids exert an action upon the insulin secreting function

Discussion

The question to be considered is whether the anti insulin and diabetogenic actions of growth hormone are of physiological importance i e whether growth hormone is a physiological blood sugar raising agent and an antagonist to insulin

Evidence was presented in previously published work[9] that an anterior pituitary hormone other than ACTH exerts a significant action in the regulation of carbohydrate metabolism It was shown that the carbohydrate metabolism of adrenalectomized and adrenalectomized gonadectomized dogs differs markedly from that of adrenalectomized hypophysectomized dogs *This difference was found to be due to an anterior pituitary factor(s)* still functioning in the adrenalectomized and adrenalectomized gonadectomized dogs Since ACTH the gonadotropins and thyrotropin were eliminated as the responsible agents it appeared very probable that growth hormone or another hormone closely linked with it is the factor participating in the regulation of carbohydrate metabolism[9]

A great amount of evidence has been obtained from *in vivo* and *in vitro* studies indicating that growth hormone has an action on carbohydrate metabolism Some of the pertinent observations were already reviewed Experiments carried out on adrenalectomized hypophysectomized dogs and reported in this paper have shown that this action of growth hormone is not due to its ACTH contaminant and can occur in the absence of the adrenocortical steroids

In view of these findings attempts were made to initiate replacement therapy with growth hormone in hypophysectomized dogs Thus far these experiments were not successful The major obstacles encountered were the development of resistance to growth hormone and the production of diabetes and toxicity Perhaps these difficulties may be avoided in the future if growth hormone obtained from pituitary glands of dogs were available Also if it were possible to prepare animals devoid of growth hormone only without exclusion of the remaining pituitary hormones then perhaps growth hormone therapy would not produce diabetes and toxic phenomena

Finally, consideration should be given to the growth hormone adreno cortical steroid relationship The C 11 17 oxycorticosteroids not only can prevent the development of growth hormone diabetes and toxicity but they can abolish or ameliorate these untoward effects produced by prior growth hormone treatment Although it appears that in the adrenalectomized dog the endogenous growth hormone exerts an anti insulin action in the absence of the adrenocortical steroids without causing diabetes or toxicity the importance of the steroid growth hormone relationship cannot be denied The experiments presented in this paper clearly reveal an important action of the adrenocortical steroids Following the intravenous infusion of glucose into hypophysectomized dogs these steroids accelerate the removal of the extra sugar from the bloodstream perhaps by increasing the secretion or release of insulin When given with growth hormone the steroids still exert this action and thereby prevent or abolish the diabetes usually produced by growth hormone

It appears then that there exists a well adjusted balance between growth hormone insulin and the adrenocortical steroids in the regulation of carbohydrate metabolism The following working hypothesis is suggested Both growth hormone and the adrenocortical steroids are blood sugar raising agents the former by inhibiting the peripheral utilization of sugar and the latter by increasing gluconeogenesis and perhaps also by inhibiting sugar utilization In this respect growth hormone and the adrenocortical steroids are synergistic and can be regarded as insulin antagonists But at the same time the adrenocortical steroids enhance the ability to secrete insulin which in turn antagonizes the action of growth hormone as regards sugar utilization The interplay of these hormones in the normal organism is so perfect that although both diabetogenic substances namely growth hormone and the adrenocortical steroids are exerting their actions the animal still does not manifest diabetes

Summary and Conclusions

1 Administration of growth hormone ameliorated or abolished the severe insulin hypersensitivity of adrenalectomized hypophysectomized dogs maintained on minimal doses of DCA

2 Concurrently growth hormone decreased the severity of the secondary hypoglycemia of the glucose tolerance tests

Thus it may be concluded that the action of growth hormone on carbohydrate metabolism is not due to its ACTH contaminant and that the adrenocorticosteroids are not essential for this action

3 Continued administration of growth hormone to hypophysectomized dogs in dosages sufficient to abolish or ameliorate their insulin hypersensitivity produced diabetes toxic phenomena or resulted in a development of species specific resistance to growth hormone With small doses of growth

still disputed action namely that they interfere with the peripheral utilization of sugar [4, 6, 8, 9, 10] could explain the changes enumerated above under 1, 2 and 3 However these actions could not explain the acceleration of the fall of blood sugar in the glucose tolerance tests It must be assumed that this is an additional action of the C 11,17 oxycorticosteroids hitherto not described This action of the steroids namely that they accelerate the fall of blood sugar in hypophysectomized dogs following intravenous glucose administration also seems to be responsible for the beneficial effects of the steroids in preventing or abolishing the growth hormone induced diabetes It is not unlikely that the adrenocortical steroids exert an action upon the insulin secreting function

Discussion

The question to be considered is whether the anti insulin and diabetogenic actions of growth hormone are of physiological importance i e whether growth hormone is a physiological blood sugar raising agent and an antagonist to insulin

Evidence was presented in previously published work[9] that an anterior pituitary hormone other than ACTH exerts a significant action in the regulation of carbohydrate metabolism It was shown that the carbohydrate metabolism of adrenalectomized and adrenalectomized gonadectomized dogs differs markedly from that of adrenalectomized hypophysectomized dogs This difference was found to be due to an anterior pituitary factor(s) still functioning in the adrenalectomized and adrenalectomized gonadectomized dogs Since ACTH the gonadotropins and thyrotropin were eliminated as the responsible agents it appeared very probable that growth hormone or another hormone closely linked with it is the factor participating in the regulation of carbohydrate metabolism [9]

A great amount of evidence has been obtained from *in vivo* and *in vitro* studies indicating that growth hormone has an action on carbohydrate metabolism Some of the pertinent observations were already reviewed Experiments carried out on adrenalectomized hypophysectomized dogs and reported in this paper have shown that this action of growth hormone is not due to its ACTH contaminant and can occur in the absence of the adrenocortical steroids

In view of these findings attempts were made to initiate replacement therapy with growth hormone in hypophysectomized dogs Thus far these experiments were not successful The major obstacles encountered were the development of resistance to growth hormone and the production of diabetes and toxicity Perhaps these difficulties may be avoided in the future if growth hormone obtained from pituitary glands of dogs were available Also if it were possible to prepare animals devoid of growth hormone only without exclusion of the remaining pituitary hormones then perhaps growth hormone therapy would not produce diabetes and toxic phenomena

Henderson of Schering Corporation for Cortate Dr A C Britton Jr of Parke Davis and Company for Thrombin Topical and Penicillin S R and Dr R K Richards of Abbott Laboratories for Pentothal Sodium Nembutal and Penicillin G Procaine Aqueous

References

1 Cotes P M Reid E and F G Young *Nature* (London) 164 209 (1949)
2 Campbell J Davidson I W F and H P Lei *Endocrinology* 46 588 (1950)
3 Campbell J Davidson I W F Snair W D and H P Lei *Endocrinology* 46 273 (1950)
4 Houssay B A and E Anderson *Endocrinology* 45 627 (1949)
5 de Bodo R C Kurtz, M Ancowitz, A and S P Kiang *Federation Proc* 9 30 (1950)
6 de Bodo R C Kurtz, M Ancowitz A and S P Kiang *Proc Soc Exp Biol Med* 74 524 (1950)
7 de Bodo R C Kurtz, M Ancowitz, A and S P Kiang *Am J Physiol* 163 310 (1950)
8 de Bodo R C and Sinkoff M W *Recent Progress in Hormone Research* New York Academic Press Inc VIII 511 (1953)
9 de Bodo R C and M W Sinkoff *Ann N Y Acad Sci* 57 23 (1953)
10 de Bodo R C Sinkoff M W Den H and S P Kiang *Federation Proc* 12 32 (1953)
11 Sinkoff M W de Bodo R C Den H and S P Kiang *Am J Physiol* 176 361 (1954)
12 Altszuler N Adams M and R C de Bodo Paper in preparation
13 de Bodo R C Earle D P Jr Schwartz I L Farber S J and E D Pellegrino *Federation Proc* 9 30 (1950)
14 Earle D P Jr de Bodo R C Schwartz I L Farber S J Kurtz, M and J Greenberg *Proc Soc Exp Biol Med* 76 608 (1951)
15 de Bodo R C Schwartz, I L Greenberg J Kurtz M Earle D P Jr and S J Farber *Federation Proc* 10 33 (1951)
16 de Bodo R C Schwartz I L Greenberg J Kurtz M Earle D P Jr and S J Farber *Proc Soc Exp Biol Med* 76 612 (1951)
17 Earle D P Farber S J de Bodo R C and M Kurtz *Federation Proc* 11 39 (1952)
18 Earle D P Farber S J de Bodo R C Kurtz M and M W Sinkoff *Am J Physiol* 173 189 (1953)
19 de Bodo R C Sinkoff M W Kiang S P and H Den *Proc Soc Exp Biol Med* 81 425 (1952)
20 de Bodo R C Sinkoff M W Kurtz M Lane N and S P Kiang *Am J Physiol* 173 11 (1953)
21 Long C N H and F D W Lukens *J Exp Med* 63 465 (1936)
22 Long C N H Katzin B and E Fry *Endocrinology* 26 309 (1940)
23 Welt I D Stetten D Jr Ingle D J and E H Morley *J Biol Chem* 197 57 (1952)
24 Katzin B and C N H Long *Am J Physiol* 126 551 (1939)
25 Thorn G W Koepf G F Lewis R A and E F Olsen *J Clin Invest* 19 813 (1940)
26 Evans G *Endocrinology* 29 731 (1941)

hormone toxic manifestations were avoided, but resistance occurred whereas larger doses produced diabetes and toxicity

Consequently, replacement therapy with growth hormone in hypophysectomized dogs was not successful

4 The administration of C 11,17 oxycorticosteroids prior to and simultaneously with growth hormone prevented the growth hormone diabetes and toxicity Furthermore, the steroids abolished or ameliorated these untoward effects produced by prior growth hormone administration

5 Untreated hypophysectomized dogs exhibited two types of glucose tolerance curves (a) in the first type the blood sugar fell rapidly from the peak values reached at the end of the glucose infusion and exhibited a secondary hypoglycemia (b) in the second type the blood sugar fell slowly from the peak value and the appearance of the secondary hypoglycemia was delayed

6 Administration of cortisone or hydrocortisone to hypophysectomized dogs raised the postabsorptive blood sugar and diminished the secondary hypoglycemia in all animals The fall of blood sugar from the peak value was greatly accelerated in dogs exhibiting the second type of glucose tolerance

7 The addition of growth hormone to the cortisone or hydrocortisone regimen of hypophysectomized dogs resulted in the following changes in the glucose tolerance tests (a) the postabsorptive blood sugar was always considerably elevated either to normal or above normal levels (b) the fall of blood sugar from the peak value remained rapid regardless of the type of glucose tolerance the animal exhibited in the untreated state and (c) the secondary hypoglycemia was completely abolished with the blood sugar remaining at normal or above normal levels

8 The insulin hypersensitivity of hypophysectomized dogs was ameliorated by the administration of C 11 17 oxycorticosteroids and was abolished on combined steroid growth hormone regimen

It is suggested that the ability of the adrenocortical steroids to accelerate the fall of blood sugar in hypophysectomized dogs following intravenous glucose infusion is due to a facilitation of insulin secretion Furthermore this action of the steroids may be responsible for their beneficial effects observed in the combined steroid growth hormone therapy

Acknowledgments

We should like to thank Mrs S P Kiang and Miss H Den for their technical assistance in these studies

We should like to express our appreciation to Drs E E Hays and I M Bunding of Armour Laboratories for growth hormone Dr R W Bates formerly of E R Squibb and Sons for growth hormone Dr E Alpert of Merck and Company for Hydrocortone Acetate and Cortone Acetate Dr K K Chen of Eli Lilly and Company for Insulin and Duracillin Dr E

followed and many of them died In 1949 at about the same time Cotes Reid and Young and our group carried out further experiments with more purified growth hormone preparations I remember that I did this work here in the United States in January with Dr Anderson From this time on we have recognized two types of hypophyseal diabetes One the transitory type I have called hypophyseal diabetes although Dr Young has called it idiohypophyseal diabetes Now we can call it idiosomatotropin diabetes as Dr Campbell is doing The other type is the permanent diabetes which I have prefixed metahypophyseal Dr Campbell has referred to it as meta somatotropin diabetes

It is very well known that the growth hormone in the rat can produce hyperplasia and hypertrophy of the islets of Langerhans We have been un able to cure diabetes in dogs as Dr Campbell also has been unable to do In rats however we have reversed the diabetes developing after subtotal pancreatectomy These animals will become diabetic for a time but giving them growth hormone results in hypertrophy and hyperplasia of the islet tissue and many of these animals lose their diabetes

That growth hormone and the diabetogenic substances are the same has *not been completely established but in every experiment to date we have* found a definite parallelism of action Yesterday Dr Wilhelmi spoke of the growth hormone of fishes Many years ago we demonstrated that prepara tions of fish pituitaries have practically no growth hormone action and no diabetogenic action in mammals We found that the dog hypophysis has both diabetogenic and growth action in the mammal More recently in the pituitary extract of whales that are very common in our Antarctic Sea we found both diabetogenic and growth action I will not mention the activities in other species because they are known by everyone here

If you use animals which have been partially pancreatectomized dia betogenic properties can be demonstrated in some other hormones of the hypophysis and in other substances not of hypophyseal origin For instance after removing most of the pancreas it is possible to obtain either transitory or permanent diabetes with thyroid and with the corticoids As Dr Best knows very well dogs are quite resistant to the cortical hormones they have no diabetes with cortisone or hydrocortisone alone Reduce the mass of the pancreas however and you can demonstrate either temporary or permanent diabetes We have recently induced metacortical diabetes in dogs We have found also that prolactin is diabetogenic In the latter experiments we have used prolactin prepared by the method of Bates and more recently a pro lactin prepared by Dr Li In partially pancreatectomized animals receiv ing prolactin we have produced diabetes in 7 of 11 dogs and in 7 of 8 cats In these experiments we have found degranulated beta cell in the islets of Langerhans of certain dogs and hydropic vacuolization in the same cells of cats which had received prolactin

The problem of purity comes up again and again whenever we speak of

27 Ingle D J and G W Thorn *Am J Physiol* 132 670 (1941)
28 Krahl M E and C F Cori *J Biol Chem* 170 607 (1947)
29 Stadie W C Haugaard N and J B Marsh *J Biol Chem* 188 167 (1951)
30 Villee C A and A B Hastings *J Biol Chem* 179 673 (1949)

DISCUSSION

Growth Hormone and Energy Sources

Designated Discussion

BERNARDO HOUSSAY The name *Growth Hormone* is a provisional name It is a historical name also because this substance was identified by experiments in hypophysectomized animals using tail growth bone growth and the increase in body weight and in epiphyseal width as the test But there are many hormones of the body which have some regulatory influence on growth Many other hormones of the hypophysis have some effect on growth Prolactin exerts an effect in the dove Thyrotropin adrenocorticotropin and the gonadotropins have actions on end-organs which in turn influence the growth process Furthermore growth is possible in mammals without the hypophysis for in the first weeks of age hypophysectomy does not interfere with growth If we know of these limitations we can continue to use the provisional name of growth hormone

A second point is that the so-called growth hormone has a regulatory action on many functions other than growth Most of these functions are metabolic but until we know more about the mechanism of action of growth hormone on these various processes we can provisionally maintain the name *Growth Hormone*

The pituitary exerts an action on carbohydrate metabolism both in normal conditions and in diabetes The suppression of the pituitary can diminish the symptoms of diabetes This we have known since 1930 And we have known also from this time that the injection of pituitary extract can again produce the symptoms of diabetes with more intensity than before From this time on the diabetogenic action of growth hormone was known In 1932 diabetes was produced both transitorily and permanently in partially pancreatectomized dogs receiving pituitary extracts The reduction in the mass of the pancreas is extremely important for the sensitization of the animal to growth hormone The animal becomes extremely vulnerable to all diabetogenic actions and I remember that in 1932 we obtained permanent diabetes in such sensitized dogs But in 1937 Young induced diabetes in non operated dogs receiving pituitary extracts This was very important for many reasons and he deserves special consideration for the accomplishment We were unable experimentally to produce permanent diabetes in 1932 primarily because the toxicity of the extract was too great After several days of injections the animals became anorexic and prostrate vomiting

into consideration the balance and interaction of the hormones It is perhaps one of the most important and difficult aspects of endocrinology

General Discussion

ERNEST KNOBIL I would like to apologize for again introducing a discordant note into this discussion particularly with reference to species differences We have studied some aspects of growth hormone effect on carbohydrate metabolism in the rhesus monkey In Figure 1 is shown the effect of the

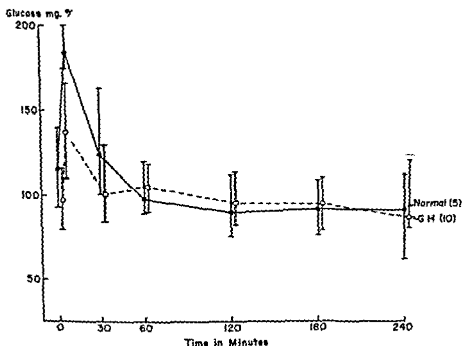


FIG 1

prolonged administration of beef growth hormone to a series of normal adult female monkeys The period of hormone administration was some 40 days at a dose level of 5 mg per kg per day The solid line depicts the glucose tolerance curve of a series of normal monkeys while the vertical line represents the ranges of response The dotted line represents the glucose tolerance curves exhibited by the female adult monkeys treated with growth hormone and the vertical line again indicates the range of response It will be noted that growth hormone in the rhesus monkey did not alter the glucose tolerance toward the diabetic type

In Figure 2 is shown the effect of growth hormone on the glucose tolerance of the hypophysectomized monkey The top line represents a composite of the glucose tolerance curves obtained from a normal group At the bottom is the glucose tolerance curve obtained in a non treated hypophysectomized

the hypophyseal hormones We cannot say definitely that the action which we attribute to the growth hormone is actually due to this hormone but to date there has been a striking parallelism Dr Reid has done much work in this direction and what we have observed is similar to the results he mentioned Dr Raben has been very kind to send me a preparation which he previously found to produce growth yet no diabetes We readily demonstrated diabetogenic properties in this preparation much as in our other growth hormone preparations Probably, this discrepancy is a matter of differences in solubility and absorption because we used a very slightly alkaline medium

Another problem in experimental diabetes which has been raised here and which Dr Long knows so well is the relationship of the adrenal to the pituitary action You are familiar with the fact that in some species adrenocorticotropin has very striking diabetogenic actions, especially in rats In other species such as the dog the growth hormone diabetogenic effect is readily manifested while in the rat growth hormone has very little diabetogenic activity In the dog both growth hormone and prolactin frequently are more diabetogenic than is corticotropin Dr Long and his collaborators in 1940 demonstrated a synergy of both hormones (adrenal steroids and pituitary growth hormone) and we have repeated this experiment in dogs with similar results It was difficult to produce diabetes in adrenalectomized dogs but this result was achieved when such an animal was partially pancreatectomized and then given the pituitary preparation Dr de Bodo mentioned that in the adrenalectomized hypophysectomized dogs he was unable to induce the diabetes with growth hormone If he used an animal with a reduced pancreatic mass perhaps he would be able to do so What he says is the repetition of what everybody was discussing some years ago about the impossibility of obtaining a diabetogenic action of anterior pituitary extracts in adrenalectomized dogs With Biasobtti and Dosne we have obtained a diabetic response in adrenalectomized dogs with subtotal pancreatectomy (extirpation of 80 to 87 per cent of the pancreas) Since reading the very important papers of Dr de Bodo about the interaction of cortisone and growth hormone in hypophysectomized dogs we have carried out the same experiment in dogs with the hypophysis intact but with a partial pancreatectomy To date the only thing which we have observed was that dogs treated with cortisone or hydrocortisone became much more sensitive to the action of growth hormone We have been unable to obtain the balance or the equilibrium in our dogs which he achieved in his hypophysectomized animals

One of the most important problems of endocrinology is defining the relationships of the hormones and for this reason the experiments of Dr de Bodo and collaborators which no one has done before are notable and they deserve a special consideration In my opinion in all these experiments on diabetes and on every problem of hormonal action we must take

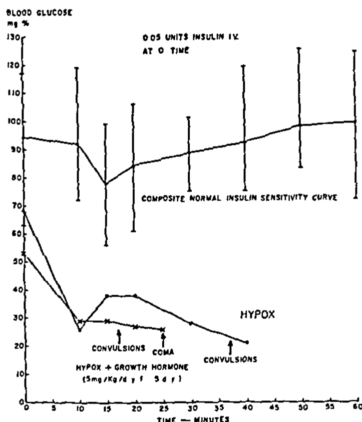


Fig 3

the hypophysectomized dog as reported by Dr de Bodo In this monkey however the administration of growth hormone at the above mentioned rate for 5 days if anything increased insulin sensitivity in that convulsions and coma appeared earlier than before hormone treatment It would be of interest to hear the comments of Dr de Bodo and Dr Lukens with reference to the role that the pancreas might play in these various responses to growth hormone in the dog and the rhesus monkey

IRBY BUNDING (The Armour Laboratories) I came here primarily to find out what has happened to some of the growth hormone I sent out and secondly to seek an answer to a problem which has been confronting us for the last several months It deals with the effects of bovine pituitary growth hormone in swine I should say now I am indebted to Dr Astwood for a comment he made in regard to specificity We have tried thyrotropin ACTH

EFFECT OF GROWTH HORMONE ON GLUCOSE TOLERANCE
OF A HYPOPHYSECTOMIZED MONKEY

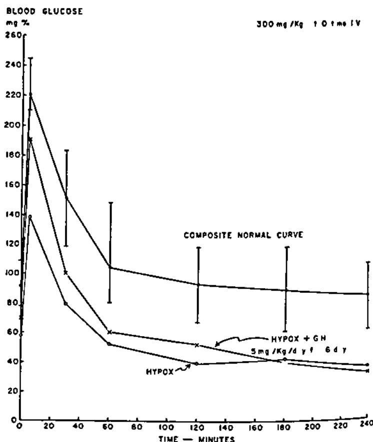


FIG 2

rhesus monkey It is quite similar to the glucose tolerance curves described by de Bodo in hypophysectomized dogs with the secondary hypoglycemia It looks a little different because the scale is not comparable Growth hormone was given in doses of 5 mg per kg a day for six days which is a very large dose compared to the level that Dr de Bodo used in his hypophysectomized dogs However this did not bring about an appreciable difference in the glucose tolerance of this hypophysectomized animal

The effect of growth hormone on the insulin sensitivity of a hypophysectomized rhesus monkey is shown in Figure 3 Again the top curve represents data of the response of normal animals to 05 unit of insulin given intravenously at zero time The vertical lines represent the range of responses Below is the curve of the response of a hypophysectomized rhesus monkey to the same dose of insulin You will note that the response to insulin of the hypophysectomized animal is quite comparable to that of

Firstly are all your corticotropin preparations active? The reason I ask this is that at the time I was working on the adipokinetic activity of corticotropin all preparations were not found active even though they were said to be so from a corticotropin point of view Secondly if these are all active preparations do the corticotropic and adipokinetic properties run essentially parallel? I might ask also whether or not your corticotropin mobilizes fat in adrenalectomized animals Dr Li has told me that this is a property of some of his pure preparations Finally do you find preparations which possess neither corticotropic nor growth effects yet have adipokinetic activity? And in conclusion I might add a minor philosophic point of my own and one which I am inclined to believe Dr Astwood would accept If I have interpreted the data correctly growth hormone corticotropin and possibly some preparation without either of these properties all have adipokinetic activity As a philosophic consideration or at least from a teleological point of view this does not sound reasonable I suspect that what we must do is to wait until the physical chemists have done a little better job and until we can deal with homogeneous molecular species

IRVING GESCHWIND We have conducted a series of experiments over the last 2 years which have been concerned with the adipokinetic effect of both corticotropin and growth hormone preparations There is one thing Dr Levin said which I would like to clarify at the present time It is to the effect that in the adrenalectomized animal neither growth hormone nor ACTH will cause an adipokinetic effect without the permissive action of cortisone or Compound F Cortisone or hydrocortisone is necessary for such action Now, Dr Levin mentioned that some older ACTH preparations did not show good adipokinetic effects in spite of the fact that their ACTH activity was vouched for Our experience with the alpha-corticotropin has been that it has a very poor adipokinetic effect when administered in an aqueous solution However when administered in beeswax peanut oil as a delaying medium it has an excellent adipokinetic effect with as little as 3 gamma being quite active in the fasted mouse On the other hand beeswax peanut oil has no effect whatsoever in enhancing the adipokinetic effect of growth hormone preparations This dichotomy is further revealed by exposing the hormones to a series of treatments such as Dr Astwood mentioned For example pepsin treatment of ACTH leaves both the corticotropic activity and the adipokinetic activity intact On the other hand pepsin treatment of growth hormone under the exact same conditions destroys both the growth hormone activity and the growth hormone adipokinetic activity In the reverse sense, chymotrypsin treatment as Dr Li pointed out yesterday leave growth hormone intact for both growth and adipokinetic activity When corticotropin is treated with chymotrypsin under the same conditions both the corticotropic activity and the adipokinetic activity are destroyed

and growth hormone in swine Of the last only we found that a dose as small as 45 mg on 2 consecutive days given to a 600 lb sow will knock her deader than a door nail Corticotropin and thyrotropin do not have this effect It is of interest to mention that in the course of our investigation we have found about 10% of the examined swine to have a pre-existing poor tolerance for a test load of glucose We thought for a while that the sensitivity to growth hormone might be associated with this It appears not to be the case We think the reaction is dependent on the age of the animal The young animal and I am taking a lead from Dr Young s book, the young swine treated with growth hormone do not respond with this highly toxic manifestation They reach a stage, however when they become susceptible and I am most curious as to what precisely is the cause of death I brought some histological slides which I showed to Dr Hartman this morning hoping he could give us perhaps a pathologist s viewpoint on what went on in the tissues There were some definite changes but he felt much more study was necessary before drawing any conclusions I am indebted to both Dr Campbell and Dr de Bodo for mentioning these toxic manifestations of growth hormone Perhaps they may prove to be very important

BERNARDO HOUSSAY The experiments on monkeys reported by Dr Knobil remind me of the results in many species resistant to growth hormone But remove part of the pancreas and usually the action of growth hormone becomes very apparent Accordingly I think it will be very important to do this in the experiments with monkeys

LOUIS LEVIN (National Science Foundation) Several questions were elicited in my mind by Dr Astwood s fine presentation and I wish to comment on his philosophic points if I may at least on one of them The first philosophic point, as I take it is where Dr Astwood lined up the various activities of growth hormone in order of the dose required to elicit the response This is a very interesting idea and he is probably on the right track However I would like to offer for consideration an amendment a point of order or something to that effect This idea of Dr Astwood s is very good provided you have lined up the activities on the basis of what actually happens under physiological conditions In other words if we remove the pituitary from an animal treat the animal with a hormone and find that the latter does a certain thing at a given dose this is not necessarily a physiological condition I presume many of the activities he has listed have been obtained more or less in this fashion Therefore the list may be subject to a revision if and when the particular activities can be lined up according to real physiological conditions

There are several questions which I would like to ask Dr Astwood

with the same technique used in Professor Collip s laboratory to demonstrate antihormones we have never found evidence of them in animals chronically treated with the growth hormone preparations then available Recently we have approached this subject from a different point of view using 2 groups of rats In group one the rats were partially fasted so they could not grow In the other the rats were fed ad lib and both groups were treated with growth hormone We found as expected that the normally fed and rapidly growing rats finally reached the plateau i e they didn t grow any more at any dose of growth hormone If at that time we made it possible for the partially starved controls to grow by giving them additional food they did so and grew rapidly even after months of pretreatment with heavy doses of growth hormone had failed It does not seem very likely that any serological reaction could account for this resistance to treatment with the growth hormone unless you assume that during the partial starvation period the mechanism of the antihormone formation had been inhibited

CHARLES BEST (Chairman) Dr Astwood reported that hypoglycemia would follow the injection of certain of his purified corticotropin preparations Was he able to get this fall in sugar in the absence of the pancreas? Does he agree with Dr de Bodo s statement that corticotropin has no extra adrenal metabolic effect?

Could I ask Dr de Bodo whether appropriate amounts of insulin would change his Type II curve into his Type I curve? The delayed curve I believe he called Type II

E B ASTWOOD I am glad that Dr Levin pointed out one objection to the business of listing hormonal effects in the order of the dose required There are a number of objections to this procedure which I am sure have occurred to some of you Certain physiological effects require much smaller dose levels than others For example the fall in ascorbic acid content of the adrenal cortex following corticotropin is an extremely sensitive response and yet another effect such as lowering the glucose tolerance may require a much larger dose But they are all part of the same phenomenon of corticotropin action We have not tested the older preparations of corticotropin recently but all the recent preparations which we have studied have been equally active We have not been able to effect a separation of the adipokinetic and the corticotropic effects

Dr Geschwind has already answered the question as to whether or not the adrenals are necessary for the adipokinetic action of corticotropin I think it was first shown by Dr Payne and confirmed by Dr Levin At least one needs to give cortisone in the adrenalectomized animals for fat mobilization to occur I am not sure there are any pituitary hormones which produce fat mobilization and which are totally free of corticotropin and growth hormone It is true that many thyrotropic preparations contain a

KARL PASCHKIS I have two questions for Dr de Bodo One perhaps is of more peripheral interest In the dogs which eventually became resistant or failed to respond to growth hormone have you tried to passively transfer that resistance? Is it possible with the serum of such dogs to do this by the old classical approach to antihormone formation? Secondly your statement was that cortisone or hydrocortisone treatment protected the animals against the diabetogenic action of growth hormone Have you done this in long term experiments or is that only in the acute experiments with the glucose tolerance test? If one gives such an animal growth hormone for a prolonged period of time will it still not develop diabetes?

C N H LONG It seems in looking at the results Dr de Bodo has presented that he has neatly balanced out the cortisone requirement of the hypophysectomized dog against the animal s growth hormone requirement He has been fortunate perhaps but I am sure he has done a lot of work in getting the right dosage The question I would like to ask is this In such a stabilized animal does an increase in the cortisone dose result in a diabetogenic action or following Dr Paschkis question a moment ago with the cortisone level constant and the growth hormone dose raised does the animal become diabetic? Our experience with this combination of hormones has been limited very largely to the rat and there as Professor Houssay mentioned it was certainly our early impression using cruder extracts of growth hormone that we got synergism in the diabetogenic effect of the cortical steroids and the pituitary factor

ABRAHAM WHITE I was going to approach Dr Paschkis first point about passive transfer perhaps by becoming interested in the syndrome which Dr Bunding described as knocking the pig deader than a door nail But it seems to me that the whole problem of foreign protein reaction in a species has not been considered It is rather striking that in the rat which forms antibodies relatively inadequately chronic injections of growth hormone for a long period of time result in a continuing response Younger animals e g young pigs which form antibodies rather inadequately cannot be knocked deader than a door nail with two successive doses of hormone whereas the older animals form antibodies readily and are much more susceptible Finally hypophysectomized or adrenalectomized animals are exquisitely sensitive to anaphylaxis whereas animals with intact pituitary adrenal systems are relatively resistant to anaphylaxis I think before we talk about the toxicity of growth hormone in terms of perhaps an inferred metabolic action we might consider that this reaction may be an anaphylaxis due to foreign protein administration

HANS SELYE In connection with Dr Paschkis question about resistance to growth hormone and its relation to antibodies I might mention that

look further for some antihormone While I am discussing this question perhaps I might answer Dr White The sick animals as far as we can see do not have any anaphylactic signs and they appear severely toxic long before resistance develops especially if the dose is large

Now going back to Dr Paschkis second question I wish to say that perhaps I didn t make it clear these are all long term experiments and our animals are receiving cortisone The minimum for this is one week it is usually several weeks Then we change to cortisone and growth hormone therapy which continues for weeks However we consider only the first two weeks of combined therapy to be of value because we wish to avoid the later period when resistance to growth hormone might develop Definitely these are all long term experiments

In reply to Dr Long s question I might say that the doses were varied I didn t mention that In most of our experiments the dose of cortisone was between 0 8 and 1 2 mg We selected a dose which we consider is more or less physiological or one which makes the adrenalectomized animal normal However we have given as much as 100 mg of cortisone to such animals and we did not induce any diabetes

In regard to the combined therapy I didn t mention it for I was worried that my time would not be adequate and I took out some slides at the last minute Of the experiments during combined therapy the blood sugar level in some animals was elevated to 110 or 120 mg per cent In these animals the dose of growth hormone was increased to 2 mg which level kills every hypophysectomized animal within 5 days and yet we obtained a very rapid drop in blood sugar So what I would like to emphasize now since I didn t have time to say it before is that in the steroid growth hormone and insulin triad there is some sort of complex interplay In some respects they are synergistic they raise the blood sugar The growth hormone interferes with the peripheral utilization of sugar the steroid increases gluconeogenesis and interferes with utilization of sugar In normal dogs we see this very clearly and we see it in such experiments as just mentioned because the blood sugar level is elevated However we believe there is another interplay—I hate to use the word antagonistic—there is another relationship in which the steroid somehow facilitates insulin secretion and thereby overcomes or prevents the hyperglycemia from growth hormone

Finally in answer to the Chairman s question it makes little difference whether the animal is of Type I or Type II response to the glucose loading The Type II animal is just as sensitive to insulin as is the Type I We did not give minimal doses of insulin to change the curves these animals are extremely sensitive to insulin

factor capable of producing fat mobilization and it does not seem to be corticotropin Whether they are completely free of growth hormone, I do not know It has been pointed out in this conference already that when growth hormone is given to an animal whose dietary intake is limited weight gain can still occur This is accomplished at the expense of fat, as first shown by Dr Milton Lee Now, under these circumstances you could regard growth hormone as being a fat mobilizing substance It is possible however that this effect is a secondary one much as if the animal were starved when the growth hormone was given

In reply to Dr Best s second question I think it must be said th t if we use the term corticotropin to mean an extract containing it then there are certainly extra adrenal effects Now with respect to Dr Best s first question about the pancreas and the hypoglycemic action of corticotropin I can say it was tried in alloxan diabetic animals even mild ones It merely served to confirm the findings of Russell and collaborators that even if the diabetes is mild there is no hypoglycemia

R C DE BODO First I would like to comment on Dr Houssay s discussion regarding prolactin We have used prolactin and have published a paper on our study Prolactin produces an anti insulin action in hypophysectomized animals and it abolishes the secondary hypoglycemia We have administered prolactin to adrenalectomized hypophysectomized dogs and thereby elimi nated the action of the ACTH contaminant which is always present Sec ondly the prolactin was heated such that the growth hormone contaminant was eliminated In these experiments we still obtained some anti insulin action This is essentially, as the matter now stands Whether or not there is one hormone the so called diabetogenic hormone mixed with prolactin and with growth hormone I couldn t answer

Now in regard to the second statement of Dr Houssay I might reply that we did not take out the pancreas partially or otherwise because we tried to establish physiological conditions as much as possible Our animals do not show glycosuria and acetonuria because they die long before the diabetes becomes that severe Many of these dogs died without a trace of acetonemia

As to Dr Knobil s problem in the monkey I have no answers I don t know why he fails to obtain any diabetogenic action in the monkey

Turning to Dr Paschkis question I will say we have done a great many experiments in regard to the resistance to growth hormone Dr Adams our Professor of Immunology tried to determine whether or not there are precipitins present He attempted passive immunization but he didn t suc ceed We ourselves took 10 ml of serum from the resistant dogs dissolved the growth hormone in the serum and administered it to dogs which never had received growth hormone That did not block the hormone action in the animal We are now about ready to take larger amounts of serum and

which were pair fed with the controls grew well for about twenty-one days and thereafter grew only very slowly for the remaining month of the treat ment It seemed to us that this reduction in growth rate of the pair fed animals reflected not a chance in sensitivity of the rats to growth hormone but rather a depletion of some endogenous substance the presence of which was necessary for growth

In an experiment designed to test this hypothesis we followed the changes in the growth rate in body composition and the respiratory quotient of both normal and growth hormone treated rats [6]

The results of this experiment showed that while growth hormone treated rats had free access to food their growth rate was almost linear and their carcase composition showed no marked depletion of the fat content On the other hand growth hormone treated rats kept on a limited food intake grew for a short time only in spite of continued injections of increasing doses of growth hormone The cessation of growth in these animals was shown to coincide with the depletion of the fat reserves of the body to about 50% of that originally present

Measurements of the respiratory quotient of the treated animals showed that while the control rats had the R Q characteristic of a mixed protein fat carbohydrate metabolism about 0 85 0 90 the growth hormone treated rats had an R Q characteristic of a metabolism based entirely on fat i e around 0 72 This low R Q was maintained by the treated rats given un limited foods for the whole of the experiment Although the R Q of the limit fed treated rats started at this low level it began to rise after some twenty days and by the end of the experiment had reached a value indis tinguishable from that of the controls Carcase analyses of these two groups of rats indicated that the fat being burned in the free fed treated rats was mainly of dietary origin but that which was being burned in the limit fed rats came largely from the fat depots

The facts I have just described suggest a close correlation between the rate of new protein synthesis and fat dissimilation in limit fed growth hor mone treated animals with protein synthesis stopping when no labile fat remains to be catabolised In animals with free access to food the extra food consumed and the energy derived from it permitted continued growth over the whole duration of our experiment In the limit fed animals how ever no extra calories can be obtained by an increase in food intake and as a consequence the fat reserves of the body are oxidised until no depot fat remains With the whittling away of this endogenous supply of calories all the dietary components must be oxidised to provide the energy for main tenance, and growth ceases

In view of this conclusion we thought it might be interesting to study the influence of growth hormone on fatty acid catabolism [7] We felt that the long term effects of growth hormone on fat catabolism were relatively clear but that the short term effect covering the change over from a mixed me

19

Growth Hormone and Fat Metabolism

A L Greenbaum
Department of Biochemistry University College, University of London

The very considerable changes in carcase composition of rats treated with anterior pituitary extracts in favour of a retention of nitrogen and water and a loss of fat first reported by Lee and Schaffer[1] and subsequently confirmed by Young illustrate the profound effect of such extracts on the metabolic pattern of treated animals The pituitary factor responsible for these changes is almost certainly growth hormone since, in 1948 Li Simpson and Evans[3] were able to report very similar changes in the body composition of rats treated with their purified growth hormone The connection between the pituitary and fat metabolism is further emphasized by the changes occurring in the proportions of the body constituents after hypophysectomy Li Simpson and Evans[3] found that hypophysectomised rats lost more nitrogen and less fat than did intact pair fed controls Further when young hypophysectomised rats are forcibly fed the same amount of food as that eaten by normal control rats they store more fat and less nitrogen than the controls [4]

There seems little question then that the anterior pituitary can exert a profound influence on the course of fat metabolism and that the factor responsible is growth hormone In fact there appears to exist a parallelism between the deposition of protein in the tissues and the disappearance of fat from the body in treated animals This dual effect of growth hormone presents us with two distinct problems The first of these is the problem of how far protein deposition is dependent on the concurrent oxidation of fat and the second is the actual mechanism by which growth hormone accelerates fat catabolism

We were interested in the first of these problems by a figure in Professor Young s 1945 paper which showed that fully fed rats injected with a growth promoting pituitary extract continued to grow in an almost linear fashion throughout the seven week period of treatment whereas those rats

Both the fatty acid oxidase system and the 2 carbon oxidation system are inhibited at 6 hours By 12 hours the fatty acid oxidase system has returned to normal and the Krebs cycle activity is stimulated above normal Actually we have found that the exact time scale of these various inhibitions and stimulations seems somewhat variable possibly due to the differing solu bilities of different batches of growth hormone or more probably to the fact that growth hormone induces two quite separate responses The meas ured effect is merely the sum of these two and the shape of the curve will vary as either the intensity or duration of either of these responses varies I shall return to this point presently But it is nevertheless true that the general pattern is always the same an initial inhibition at the shorter time interval followed by the rise at the longer time interval

In order to make sure that the difference in response at 6 hours and 12 hours was not simply due to a slow rate of absorption of the growth hor mone giving a much higher level of the hormone in the blood at 12 hours Dr Ottaway kindly measured the level of growth hormone in the plasma 6 hours and 12 hours after the injection of 0 5 mg of growth hormone in traperitoneally In Table 1 is shown the result of this assay

Table I

CONCENTRATION OF GROWTH HORMONE IN THE PLASMA OF RATS INJECTED WITH GROWTH HORMONE*
(from Ottaway unpublished work)

Controls	*6 Hrs after Injection*	*12 Hrs after Injection*
0 05	3 80	0 54
0 06	3 50	0 43
	3 05	
Mean 0 055	3 45	0 50

* All values in μg /ml plasma

As you can see he found that the 12 hour level was much less than that found at 6 hours which eliminates the possibility that the response at 12 hours was simply due to a rising tide of growth hormone in the plasma

Considering this experiment as a whole two interesting points emerged Firstly there is a rather surprising two phase response in so short a time as 12 hours Secondly there is the problem of the rate of acetoacetate produc tion We have calculated that the liver of a rat treated with growth hormone for 2 to 3 days produces somewhere around 150–200 mg more aceto acetate per day than does the control liver and yet we have found only some 3–4 mg ketone bodies in the urine The problem is therefore where this extra acetoacetate is oxidised

We first investigated the two phase reaction For several reasons it seemed to us likely that the response at 6 hours and that at 12 hours could be due

tabolism to one based predominantly on fat would be more instructive Accordingly we studied the course of the breakdown of octanoic and oleic acids in the livers of rats treated with growth hormone for relatively short times By measuring the oxygen uptake in the presence and absence of fatty acid substrates and the acetoacetate production we were able to calculate the rate at which fatty acids are degraded as far as the 2 carbon stage and the rate at which the 2 carbon units are oxidised to CO_2 and water

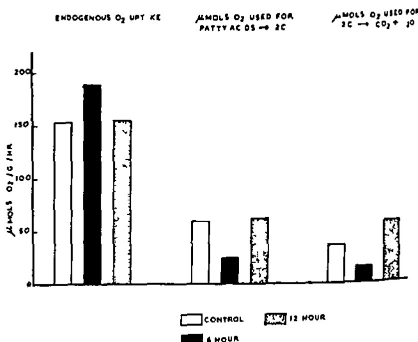
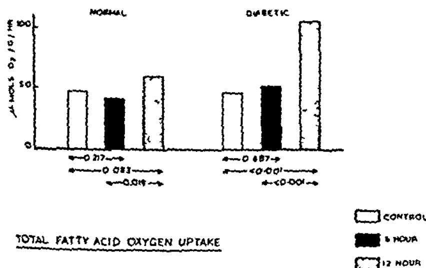


FIG 1

We found that the most striking changes in fat metabolism occurred in the first 12 hours after the injection of growth hormone At 6 hours there is an increase in the endogenous oxygen uptake but a remarkable decline in the oxygen used for fat catabolism Acetoacetate production is also inhibited at this time interval After 12 hours the endogenous oxygen uptake had returned to normal and the oxygen used in fat oxidation had increased until it was not significantly greater than the control level When the oxygen used in fat catabolism is partitioned into two steps (a) the oxidation of fatty acids by the fatty acid oxidase system as far as the 2 carbon fragment formation and (b) the oxidation of the 2 carbon fragments by way of th Krebs tricarboxylic acid cycle to CO_2 and water, a similar pattern is revealed (Fig 1)



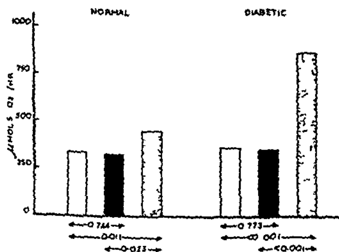


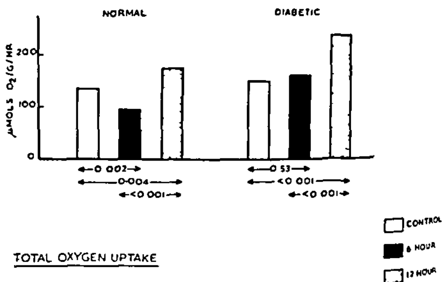
FIG 3

per cent Both the normals and diabetics were divided into three groups one group consisted of the controls and two groups were injected with 0 5 mg growth hormone following which one group was killed at 6 hours and the other at 12 hours As before we measured the endogenous oxygen uptake the oxygen attributable to fatty acid catabolism and acetoacetate formation The results of this experiment are shown in Figures 2 3 4 and 5

I should just like to remind you that the total oxygen referred to here excludes the endogenous respiration and represents the combined oxygen utilized in oxidising the fatty acids to 2 carbon fragments and in subsequently

to the combined action of two contra acting hormones probably insulin predominating in the first few hours and growth hormone subsequently We decided therefore to investigate this problem by contrasting the response of normal and diabetic rats to a single intraperitoneal injection of growth hormone

In this experiment we used alloxan diabetic rats which had been kept 21 days after the induction of diabetes to make sure that they were free of endogenous insulin Their blood sugars varied between 400 and 600 mg



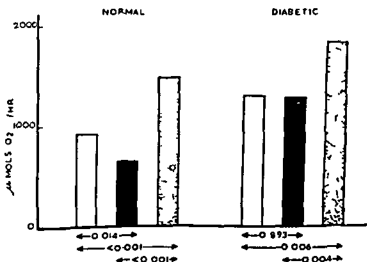


FIG 2

There appears to be no inhibition of the fatty acid oxidase in either normal or diabetic rats 6 hours after growth hormone but there is a stimulation of this system at 12 hours The stimulation is much more striking in the diabetic animals than in the controls In Figure 4 are shown the changes in the rate of oxidation of the 2 carbon fragments to CO and water

Here again there is an inhibition of activity in normal rats at 6 hours which is not paralleled in diabetic animals and there is the further difference in this case at 12 hours where the normal rats have recovered their activity but the diabetic animals have begun to be inhibited In Figure 5 are

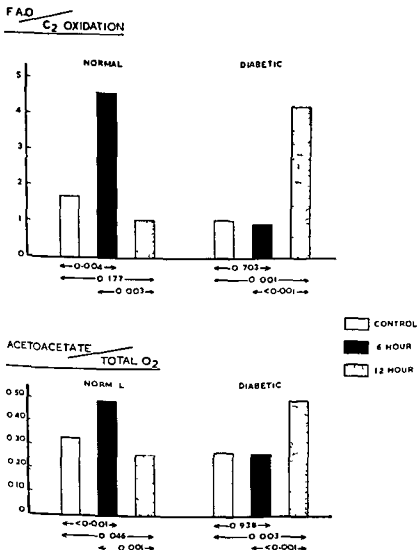
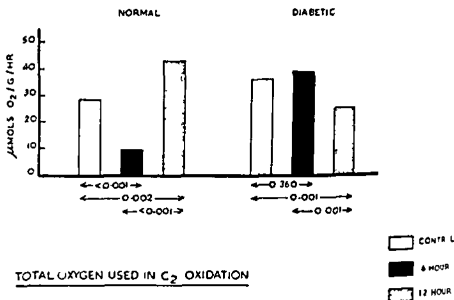


FIG 5



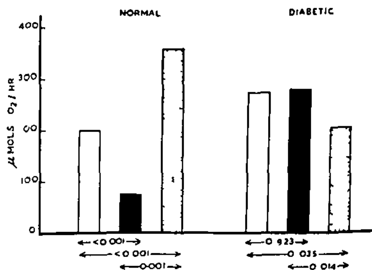


FIG 4

oxidising the 2 carbon fragments to CO_2 and water I might point out also that the inhibition of oxygen uptake in normal animals 6 hours after growth hormone treatment is not found in diabetic animals and that the stimulation of oxygen uptake at 12 hours is found in both groups This gross oxygen uptake can be partitioned between the two phases of fat catabolism and in Figure 3 is shown that part of it which is used in the formation of the 2 carbon units

take liver slices from either control rats or from rats pre treated with growth hormone and incubate them in Krebs Ringer bicarbonate solution containing the radioactive pyruvate at a level of 100 microcuries per litre of medium Carrier pyruvate is added to give a final substrate concentra tion of 0 02*M* After a three hour incubation the total CO is collected the liver slices are taken and the fatty acids extracted In the breakdown of pyruvate there is an initial decarboxylation resulting in a radioactive 2 carbon unit which is then available amongst other things either for oxidation to CO_2 and water or for fat synthesis The activity of the respired CO_2 is a measure of the rate of oxidation of this 2 carbon unit and the activity of the isolated fatty acids is a measure of the rate of its incorporation into fat In Table 2 is a summary of an experiment of this type

Table 2

THE METABOLISM OF 2-C^{4} PYRUVATE IN GROWTH HORMONE TREATED RATS

TREATMENT	*CO* *Total Activity* *Counts per Min*	*FATTY ACIDS* *Specific Activity* *Counts/min /mg carbon*
Control	$1\ 0 \times 10^5$	2835
Experimental Injected 0 5 mg growth hormone 6 hrs previously	$1\ 20 \times 10^5$	239
Experimental Injected 0 5 mg growth hormone 12 hrs previously	$1\ 43 \times 10$	117

You can see that the injection of growth hormone increases the rate at which pyruvate is being oxidised to CO and water You can see also the very powerful inhibition of fat synthesis in growth hormone treated animals I must confess that the experiment in Table 2 was the most dramatic result we have had so far The severity of the inhibition at 6 hours is rather sur prising in view of our previous conclusion that the animal was predominantly under the influence of insulin at this time interval But what it does indicate perhaps is that growth hormone is so powerful an inhibitor of fat synthesis that it can exert its effect even in the presence of insulin which is known to have the opposite effect We must remember of course that the growth hormone was injected into an intact animal and there could have been an adrenal effect Welt and Wilhelmi[10] have shown that the adrenal hormones also inhibit fat synthesis

summarized the last three which illustrates how very different is the metabolism of growth hormone treated animals in the presence and absence of insulin The top half shows how the oxygen uptake is partitioned between the fatty acid oxidase system and the 2 carbon oxidation system

In normal animals the supply of 2 carbon units at 6 hours exceeds the capacity to oxidize them and there is a resultant ketosis Later the capacity for 2 carbon oxidation is recovered and the 2 carbon fragments instead of being shunted off to form acetoacetate, are oxidized in the Krebs cycle In diabetic animals the enzyme systems seem fully able to cope with the fatty acids at 6 hours but at 12 hours the 2 carbon oxidation system seems deficient and ketosis supervenes

The data presented in the last four figures help to throw some light on the two phase response of fat metabolism to grow hormone The first phase is dependent to a large extent, on a functional pancreas while the second phase seems more independent of it Many workers and in particular Milman and Russell[8] have suggested that the injection of growth hormone causes a release of insulin which produces its characteristic effects for a period of up to 8 hours From the evidence I have presented I should like to suggest that the first response of fat metabolism to growth hormone is largely due not to growth hormone itself but to insulin released by it either from the pancreas as suggested by Milman and Russell [8] or from the tissues as suggested by Ottaway [9] Thus at 6 hours the liver is predominantly under the influence of insulin which would account for the increased endogenous respiration and the decreased fat degradation It would also account for the reduced oxidation of fatty acid 2 carbon units in the Krebs cycle which is presumably fully engaged oxidising the 2 carbon fragments derived from carbohydrate metabolism After 8 hours or so the insulin effect diminishes and the true growth hormone pattern which so far had been masked becomes apparent Thus the second phase in our experiments is in fact the true growth hormone effect an effect which is characterised by an increased fatty acid oxidase activity and an increased ability to oxidise the resultant 2 carbon fragments through the cycle

In view of the results we obtained when measuring the effect of growth hormone on the enzymic degradation of fatty acids we thought it might be interesting to study the reverse reaction as well that is the effect of growth hormone on the synthesis of fatty acids Such a study in fact has been made *in vivo* by Welt and Wilhelmi[10] who measured the rate of fat synthesis in rats injected with D O over a period of from one to eight days The work described here was done *in vitro* at very short time intervals I would like to make it clear at the outset that these experiments which are being done in collaboration with Dr Glascock of Reading are decidedly preliminary and are very incomplete but they are sufficiently interesting nevertheless to merit reporting here

The substrate we have been using is 2 C^{14} pyruvate The procedure is to

There is one last experiment I would like to report and this concerns the level of oxidised and reduced diphosphopyridine nucleotide (DPN) in liver In the last year Lynen and Ochoa[11] have put forward a scheme for the enzymic mechanism by which fats are synthesised and degraded (Fig 6)

Of particular interest to us was β keto reductase which is a DPN linked enzyme bringing about either the reduction of keto acids to the corresponding hydroxy acid in the presence of DPNH or the oxidation of the hydroxy acid to the keto acid in the presence of DPN Whether the enzyme favours

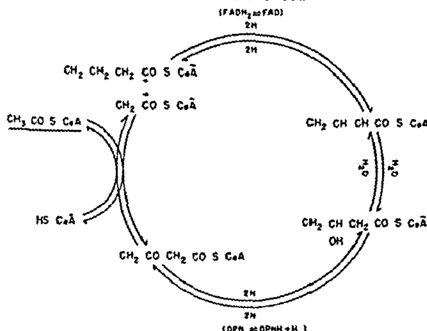


FIG 6

the reduction i e fat synthesis or oxidation i e fat degradation depends on the availability of DPN or DPNH Indeed Lynen has said[14] It would appear that in the living cell the DPNH DPN ratio may determine whether synthesis or degradation of the carbon chain will occur It seemed to us most important therefore to know what changes occurred in the levels of the oxidised and reduced DPN in the liver after growth hormone treatment Accordingly we measured the coenzyme by a modification of the method of Holzer Goldschmidt Lamprecht and Helmreich[15] in the livers of growth hormone treated rats and the results are presented in Table 3

I would like to draw your attention to only those columns showing the total liver DPN or DPNH and to the column showing the ratio of DPN to DPNH The study of the levels of coenzyme per gram of tissue are not very

Nevertheless, we feel that the severity of the inhibition makes it unlikely that the small amount of ACTH impurity, possibly present in our purified growth hormone, could have been the causative agent although a possible synergistic effect between growth hormone and adrenal hormones cannot be ruled out

There is another factor of course which contributes to the low specific activity of the fatty acids in the liver of rats treated for 6 or 12 hours and this is the dilution effect caused by the mobilisation of fat to the liver from the depots While this undoubtedly will cause a reduction in the specific activity it could not have caused the ten fold decrease actually observed

As I said this is a very preliminary account and we shall certainly extend this work to cover the possibilities I have mentioned We shall also repeat this work with 1 C^{14} pyruvate to get a measure of the effect of growth hormone on the formation of the 2 carbon fragments

I mentioned earlier the problem of the site at which the extra acetoacetate formed in the liver was metabolised We sought to answer this problem by studying the activity of the acetoacetate activating enzyme in the muscles By using a system modified from that described by Green Goldman Mii and Beinert[11] we succeeded in measuring the activity of the acetoacetate activation and cleavage enzyme in muscle We found no difference at all in the activity in muscles from rats treated with growth hormone for 3 days as compared with those from normal controls There could be several reasons for this failure to find a difference Firstly it is always possible that there is no difference between the muscles and that the site of the extra metabolism is not in the muscles at all but in the other extrahepatic tissues notably the kidneys and heart But there is another explanation which is at present engaging our attention

We have used an assay procedure which estimates the level of only the enzyme in the tissue since we add all the necessary cofactors in excess and it is quite possible that it is not the level of the enzyme which changes but the availability of these cofactors As you know the activation of aceto acetate is achieved by the transfer to it of coenzyme A from succinyl CoA In our system excess succinyl CoA was generated by the oxidation of α keto glutaric acid by purified α keto glutarate oxidase It could well be that the rate limiting step in acetoacetate activation is not the enzyme itself but the supply of succinyl CoA or even perhaps of CoA itself In this last connection it is interesting that Lotspeich[1] has found that growth hormone treated rats develop a pantothenic acid deficiency and pantothenic acid is a necessary precursor for Coenzyme A It is possible that the increased requirement is needed to keep pace with an increasing rate of coenzyme synthesis

We plan to measure several other of these CoA dependent enzymes and if the same kind of result is obtained we shall have to extend our investigation to a study of the cofactor requirements in the tissues of treated rats

results and ours This point will require further investigation Nevertheless we seem to have found a very different state of affairs in growth hormone treated rats than obtains in normals

It is not possible to account for the high level of DPNH as resulting from a decreased activity of either β keto reductase or even of the whole Lynen cycle Since the cycle is fully reversible and since we know from previous experiments that fat catabolism is proceeding at a high rate it follows that the whole cycle is fully functional We must conclude I think that growth hormone acts on an enzyme or an enzyme system outside this cycle If the direction of the cycle is governed by the availability of substrates or removal of end products we can suggest that the cycle is reversed by a growth hormone induced imbalance such that either the supply of intermediates from fatty acids or the removal of intermediates by β keto thiolase is stimulated The high level of DPNH in our treated rats can be explained quite simply as being the result of fatty acid catabolism and not in any way as a driving force for fat synthesis Thus with each catabolic turn of the cycle two hydrogens are removed from the hydroxy acid to produce the keto acid and this reaction is linked to the reduction of one molecule of DPN to DPNH The interest of this particular experiment is that it directs our attention away from the Lynen cycle as a possible site of growth hormone action towards those enzymes which lead to the activation of the fatty acids prior to their entry into the cycle As a preliminary attempt we are now assessing the effect of growth hormone on the first of these enzymes which was described by Kornberg and Pricer[17] as activating the long chain fatty acids

References

1 Lee M O and N K Schaffer *J Nutrition* 7 337 (1934)
2 Young F G *Biochem J* 39 515 (1945)
3 Li C H Simpson M E and H M Evans *Growth* 12 39 (1948)
4 Samuels L T Reinecke R M and K Baumann *Endocrinology* 33 87 (1943)
5 Levin L *Am J Physiol* 141 143 (1944)
6 Greenbaum A L *Biochem J* 54 400 (1953)
7 Greenbaum A L and P McLean *Biochem J* 54 413 (1953)
8 Milman A E and J A Russell *Endocrinology* 47 114 (1950)
9 Ottaway J H *Brit Med J* II 357 (1953)
10 Welt L D and A E Wilhelmi *Yale J Biol Med* 23 99 (1950–51)
11 Green D E Goldman D S Mii S and H Beinert *J Biol Chem* 202 137 (1953)
12 Lotspeich W D *Proc Soc Exp Biol Med* 73 85 (1950)
13 Lynen F and S Ochoa *Biochim et Biophys Acta* 12 299 (1953)
14 Lynen F *Harvey Lectures* 1952–53 p 210
15 Holzer H Goldschmidt S Lamprecht W and E Helmreich *Hoppe Seyler's Z physiol Chem* 297 1 (1954)
16 Helmreich E Holzer H Lamprecht W and S Goldschmidt *Hoppe Seyler's Z physiol Chem* 297 113 (1954)
17 Kornberg A and W E Pricer *J Biol Chem* 204 329 (1953)

Table 3

COENZYME I LEVELS IN THE LIVER OF GROWTH HORMONE TREATED RATS

Group	*No of Animals*	*Per g Wet Tissue*		*Total Liver*		*Total Liver DPN + DPNH*	*DPN / DPNH*
		DPN	*DPNH*	*DPN*	*DPNH*		
Controls	10	284 ±16 2	118 ±5 2	1864 ±113	775 ±33	2639 ±142	2 41 ±0 08
Rats injected with growth hormone 6 hrs previously	5	292 ±7 6	158 ±12 7	2209 ±98	1203 ±98	3412 ±154	1 87 ±0 15
Rats injected with growth hormone 12 hrs previously	5	302 ±18 9	157 ±15 0	2180 ±84	1138 ±112	3318 ±147	1 96 ±0 15
Fishers P Control v 6 hours		0 760	0 005	0 075	0 001	0 006	0 008
Control v 12 hours		0 509	0 007	0 094	0 001	0 011	0 015
6 hours v 12 hours		0 632	0 946	0 878	0 675	0 674	0 666

helpful because the livers from the hormone treated rats are infiltrated to a variable degree with mobilised fat

I would like to point out first that there was an actual synthesis of total coenzyme I after growth hormone treatment This may well reflect a general pattern of growth hormone action increasing the level of rate limiting or rather reaction directing cofactors and not increasing the levels of the enzymes themselves This is a point which could bear further examination Another striking point which emerges is that although the level of DPN remained more or less unchanged a considerable increase occurred in DPNH a change large enough to be statistically significant and to make a significant change in the DPN DPNH ratio The odd thing about this result is that it ran counter to the action postulated by Lynen in normal rats i e we observed a high level of DPNH associated with a decreased fat synthesis

Helmreich Holzer Lamprecht and Goldschmidt[16] have found a correlation between a reduced level of DPNH and an increased acetoacetate production in both starved and diabetic rats In our experiments we found a raised DPNH at a time when acetoacetate formation seems to have been inhibited and there seems to be some correlation therefore between their

free of appreciable growth hormone contamination to exhibit a high grade of ketogenic activity in the rat[1] This was particularly noteworthy since it had already been shown that cortisone and hydrocortisone inhibited fasting ketosis while adrenalectomy enhanced it [3] Hence the production of ketosis by way of the corticotropic property of the hormone seemed hardly likely As will be discussed later the latter mechanism was easily ruled out by the demonstration that the ketogenic activity of ACTH persisted in the adrenalectomized rat Subsequently using a standardized technique for the detection of ketogenic activity we examined various samples of so called purified pituitary hormones in order to determine what correlation there was between ketogenic activity and the label on the preparation (Table 1) All

Table 1

KETOGENIC ACTIVITY OF PITUITARY PREPARATIONS*

Sample	*Dose in mg*	
	Minimum Active	*Maximum Inactive*
Astwood Oxycel ACTH	0 030	0 003
Wilson Oxycel ACTH (82002)	0 030	0 003
Wilson Oxycel ACTH (86890)	0 030	0 003
Wilson Oxycel ACTH (90834)	0 030	——
Wilson Commercial ACTH (84364)	0 100	——
Armour Growth—3PKR 3	0 100	——
Armour Growth—22KR 1	0 300	0 100
Horner Growth—C 35 38 1 (Wilhelmi)	——	0 300
Wilhelmi Growth—B169GH	1 000	0 600
Wilhelmi Growth—B111 60GH	1 000	0 100
Wilson Growth ST H 2 (Raben)	0 800	——
Horner Growth PR 1 (Raben)	4 000	2 000

* This table represents only the smallest active and the largest inactive doses *tested* and therefore is only a crude estimate of the ketogenic activity of the pituitary preparations studied

tested samples of ACTH and growth hormone exhibited ketogenic activity but there was great variability in potency In general the samples of ACTH were the most active only an Armour growth hormone preparation 3PKR 3 approached them in this respect The oxycel purified corticotropins were the most potent in inducing ketosis Although simultaneous assays for growth activity were not performed and hence the most suitable base line for comparison of growth and ketogenic activities was missing several of the growth hormone samples tested were known to have high growth activity (Armour 3PKR3 22KR1 Wilhelmi B169GH and B111 30GH) Accordingly the data raise the question as to whether or not growth and ketogenic activity are necessarily properties of the same hormone

Further information on this point has been derived from a study of the comparative metabolic properties of a growth hormone sample PR 1

20

Factors Involved in the Ketogenic Action of Growth Hormone*

Frank L Engel

Departments of Medicine and Physiology Duke University Durham N C

The problem of the relationship of the anterior pituitary gland hormones to ketosis has fascinated investigators for more than twenty years So far it has defied solution Despite substantial progress in our knowledge of intermediary metabolism and in the purification of pituitary hormones at the present time we are still confronted by the same basic questions which have faced all past investigators interested in this problem (1) Is the principle concerned with ketosis separate and distinct from all other pituitary hormones or is the ketosis a by product of the metabolic action(s) of one or more of the well known pituitary hormones? (2) What is the precise mechanism by which intermediary metabolism is modified by the pituitary to result in ketosis?

Originally our interest in this problem was concerned chiefly with the second question but as our studies progressed it became increasingly apparent that the understanding of the hormonal actions in ketosis must await the identity of the hormone Most investigators have tended to identify the ketogenic factor of the anterior pituitary with growth hormone and even those who have argued that it is a separate principle have sought it in extracts which contain growth hormone rather than other known pituitary factors It came as something of a surprise when during a study of the role of the adrenal cortex in ketosis we found samples of ACTH presumably

* Supported by research grants from the American Cancer Society administered by the Committee on Growth of the National Research Council the Eli Lilly Laboratories Indianapolis Indiana the Duke University Research Council and the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health Public Health Service (A 14-) and by Contract No DA-49 007 MD 134 with the Research and Development Division Office of the Surgeon General Department of the Army

being kept in the refrigerator For all other tests the solutions were prepared just prior to use and as follows

1 Suspended in saline and brought into solution by adding glacial acetic acid to pH 3 5 Complete solution was not usually achieved

2 Same as above but suspended in a vial in a boiling water bath for 30 minutes prior to use

3 Dissolved in 0 1N NaOH and immediately brought to pH 9 5 by the addition of 0 2N HCl

4 Dissolved in 0 1N NaOH and allowed to stand overnight (16–20 hours) either in the refrigerator or at room temperature (25° C) (The tests for ketogenic and adipokinetic activities were done on the pituitary extract kept at room temperature those for growth diabetes and hyperglycemia on the extract at 4° C) The solution was adjusted to pH 3 5 with 0 2N HCl for injection

The results of this study are shown in Figure 1 where it can be seen that preparation PR 1 in saline at pH 3 5 exhibited all five of the activities tested for Heating the hormone solution at pH 3 5 in a boiling water bath resulted

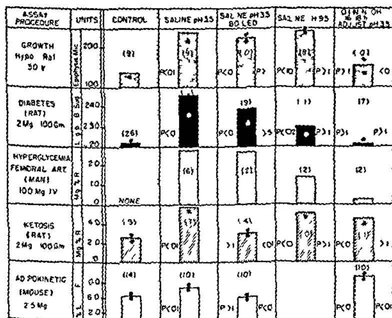


FIG 1 Comparative metabolic activities of growth hormone (Horner PR 1 Raben Westermeyer) See text for description of test procedures The control column represents the response in the respective tests by untreated animals The second column shows the data for the standard preparation of the pituitary extract prior to chemical treatment The P values to the left of the column refer to statistical significance compared to corresponding untreated controls those to the right compared to the standard preparation (column 2)

which was prepared by the method of Raben and Westermeyer[1] and supplied in generous quantities by Dr L Mitchell of Horner and Company Ltd Montreal The hormone was tested by the following techniques (1) *Growth activity* This is a measure of its influence on the tibial epiphysis of the young hypophysectomized rat The results are expressed as the width of the epiphysis after four days of treatment with 50 micrograms of hormone daily compared to saline treated hypophysectomized controls (2) *Induced diabetes* This technique which will be described in detail elsewhere, depends on the induction by growth hormone of hyperglycemia and glycosuria in the rat force fed a high carbohydrate diet and primed with a subdiabetogenic dose of cortisone [6,7] The index of diabetes is the logarithm of the pooled values of mean blood sugar levels two hours after feeding on the 4th and 5th days of combined treatment with growth hormone and cortisone compared with that of controls receiving cortisone alone (3) *Hyperglycemia in man* In normal human subjects the intravenous infusion of 60–100 mg of preparation PR 1 in 500 ml saline in 30 minutes results in a prompt rise in the femoral arterial and hepatic venous blood sugar This occurs without any change in splanchnic blood flow urea or amino nitrogen balance and, hence is due to an outpouring of glucose from the liver presumably by glycogenolysis [8,9] Hyperglycemia subsides soon after discontinuing the infusion The criterion used in this test was the maximal rise in femoral arterial blood sugar observed during the intravenous infusion of PR 1 This response is reminiscent of that described by Bornstein Reid and Young[10] and Foa[11] in animals and attributed by them to a stimulation of the secretion of the hyperglycemic factor of the pancreas (glucagon) (4) *Ketogenic activity* Male rats weighing 180–250 grams and at the end of a 16–20 hour fast received the test material intraperitoneally after being anesthetized with 4 mg of nembutal per 100 grams of body weight Blood was taken from the tail prior to treatment and the animals placed in a constant temperature incubator at 33 C for 3½ hours and under light anesthesia [1] The rise in the blood ketone level in 3 5 hours in the treated rats was estimated using a modification[1] of the techniques of Greenberg and Lester[13,14] and Michaels et al [15] and was compared to that of untreated controls As is discussed elsewhere the nembutal anesthesia and the constant environmental temperature are important factors of this test the sensitivity of the anesthetized rat to the ketogenic factor being as much as 100 fold greater than that of the unanesthetized rat [1] (5) *Adipokinetic activity* Liver fat was determined by the method of Handler[16] 7 hours after the intraperitoneal injection of the pituitary preparation in female mice which were allowed access to food up to the time of injection The controls received an equal volume of saline The assay technique was essentially that described by Rosenberg [17]

In an attempt to dissociate these activities one from another the pituitary extract was prepared in the following fashions For the growth and diabetogenic assays the hormone was made up and used for a 4 or 5 day period

resistant to alkali treatment and destroyed by boiling in acetic acid The original observations with oxycel corticotropin were made with samples provided some four years ago through the courtesy of Dr David Klein of the Wilson Laboratories Subsequently a series of publications appeared from the laboratory of Dr E B Astwood[17 0 21] which also lent support to the concept that this material exhibited marked metabolic activity, including adipokinetic ketogenic R Q depressing and hypoglycemic activities and these could not be attributed to its adrenocorticotropic action per se These data have been described elsewhere in this volume and, hence will not be reviewed here[2]

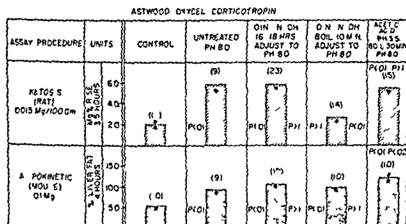


Fig 2 Comparative metabolic activities of Astwood oxycel corticotropin

Through the kindness of Dr M Raben a quantity of oxycel corticotropin was made available for study by the techniques just described This preparation was dissolved in saline adjusted to pH 8 0 with 0 1N NaOH and injected at a dose of 0 015 mg per 100 g of body weight for the ketosis test in the rat and 0 100 mg per mouse for the fat mobilization test In the latter test liver fat concentration was measured four hours after the injection instead of seven hours as was done in the previous study The hormone otherwise was treated similarly to preparation PR 1 except that it was always brought to pH 8 0 prior to injection In Figure 2 are presented the results The material exhibited high ketogenic and adipokinetic activities, and both activities persisted after exposing the material to alkali for 16–20 hours Since Rosenberg[37] reported a decline in adipokinetic activity on boiling such preparations for 2–3 minutes at pH 11 0 another series was run in which the corticotropin was boiled in 0 1N NaOH for 10 minutes As seen in Figure 2 this resulted in complete loss of ketogenic but not of adipokinetic activity The differential loss here may be related to the fact that ketogenic activity was tested at close to the minimal effective dose and hence partial loss would present as complete disappearance of activity In contrast to

in loss of ketogenic and adipokinetic but not of growth, diabetogenic and hyperglycemic activities Alkali treatment overnight yielded just the opposite results, with loss of growth diabetes and hyperglycemia and retention of adipokinetic and ketogenic responses The liver fat response was actually increased significantly in the alkali treated extract Injection of the extract at pH 9 5 instead of 3 5 did not influence the results This is of some interest in view of Reid s observation that Raben Westermeyer growth hormone was less active in producing diabetes and growth[18] when injected at an acid pH

The most noteworthy feature of these results is the fact that growth diabetogenic and hyperglycemic activities and ketogenic and adipokinetic properties respectively each behaved as a group in their response to the chemical treatments These results suggest that the above two groups of activities might be separated but this conclusion must be tempered by the appreciation that the assay methods used have unknown and undoubtedly different degrees of sensitivity It is entirely conceivable, for example that a uniform decrease in all activities might present as no response in one test and a detectable reaction in another assay procedure However the fact that the responses were opposite on acid and alkali treatment make this interpretation unlikely In addition the following data may be recorded also as arguments against the above view In the growth assay a 12 microgram dose gave a good tibial response and hence one must assume that there had been at least 60 per cent inactivation by alkali treatment to result in a complete loss of response in the tibial test The diabetogenic assay gave a positive response at 1 0 mg per 100 grams of body weight and hence the loss of activity with alkali must be presumed to have exceeded 50 per cent Since PR 1 contained no ketogenic activity at a dose of 1 0 mg per 100 grams of body weight and no adipokinetic response at a dose of 1 25 mg in both cases a 50 per cent reduction of dosage over that recorded in the chart it is difficult to conceive that a great reduction in general hormone activity had in fact occurred without being detected in the latter two tests

It should be noted that according to the manufacturer s assay preparation PR 1 was contaminated with small amounts of ACTH and TSH In addition Welt[19] has found it to possess antidiuretic activity No assays for any of these hormones were made in this laboratory Data will be presented below to show that ACTH per se is not responsible for the ketosis Pitressin was found to have no effect on hepatic glucose output in man No information is available as to whether TSH was concerned with the adipokinetic and ketogenic responses in this study but this does not detract from the evidence against growth hormone being the effective agent

Since oxycel purified ACTH was found to be a much more active ketogenic material than PR 1 and other available growth hormone preparations it was of obvious importance to determine whether the adipokinetic and ketogenic properties of this particular hormone preparation, likewise was

one and reputedly did not have growth promoting properties Anselmino and Hoffman [7, 8, 9] claimed a separation of ketogenic activity by dialysis which was most effective at an alkaline pH but this observation was not confirmed by other workers Collip and his co workers also had evidence for a separation of these two activities [30] Shipley and Long in 1938[31] carried out assays for growth promoting properties ketosis producing and diabetogenic activities ACTH TSH and prolactin in pituitary extracts treated in different fashions They came to the conclusion that the ketosis could be adequately accounted for as a secondary response to the influences of growth hormone on carbohydrate and protein metabolism since they could not separate these activities by any techniques they tried However Harrison and Long[3] noted two years later that when a saline anterior pituitary extract active in nitrogen retention and ketosis was incubated overnight at 4° C in N/15 NaOH nitrogen retention activity was lost while the ketosis response persisted From this observation they drew no conclusions concerning the identity or non identity of the nitrogen retaining and ketogenic activities

The recent studies of Li and Papkoff[33] and of Ellis et al [34] on the properties of so-called purified growth hormone prepared by different techniques may also have some bearing on this problem Both groups of investigators have found evidence for inhomogeneity of pituitary growth hormone when treated with alkali an increasing loss of growth activity resulting as exposure to alkali is prolonged Li and Papkoff found complete loss of growth activity after the hormone was exposed to 0 1N NaOH for 24 hours at 0° C and for 6 hours at 25 C At alkaline pH levels the growth hormone separated into a fast and slow moving component The former could be separated out by Ellis et al who found it to have no growth promoting activity It would be of great interest to test this component for other pituitary metabolic activities particularly ketogenic and adipokinetic

The suggestion from these studies that growth and adipokinetic activities may be separable need not have any special implications with respect to the native state of the hormone in the pituitary gland since it is conceivable that alkali treatment might result in the splitting off of an active prosthetic grouping from the parent protein On the other hand it does have important implications concerning the primary site(s) of metabolic action of these fractions if the two sets of activities can be shown to occur independently This does not obviate the possibility that under normal circumstances their metabolic actions are complementary or synergistic as is the case with growth hormone and insulin and with growth hormone and thyroid

Since all the data so far presented have indicated that the high ketogenic and adipokinetic activities of oxycel corticotropin have been derived from studies on intact animals it is appropriate to present the evidence at this time that *this response is not mediated by a ketogenic factor from the*

PR 1 which lost ketogenic and adipokinetic activities when boiled at pH 3 5 oxycel corticotropin withstood this treatment without loss

At the inception of the studies with oxycel corticotropin it was felt that they had special significance with respect to the contention that the ketogenic factor was not identical with growth hormone since the oxycel ACTH was thought to be free of growth promoting activity However Russell [3] using the nitrogen retention test for growth hormone described earlier in this meeting [4] finds that this property is present in oxycel corticotropin and hence one cannot assume it to be free of growth hormone This observation seriously damages the contention that the manifold metabolic activities of oxycel corticotropin which have previously been associated with growth hormone activity actually represent activities of a hormone distinct from growth hormone Our finding of loss of growth diabetogenic and hyperglycemic activity but retention of ketogenic and adipokinetic activities on alkali treatment suggested another possible separation which needed to be evaluated by Dr Russell s technique Preliminary results by Dr Russell with oxycel ACTH and some samples of Wilhelmi growth hormone have indicated that nitrogen retention is still demonstrable after treatment of the preparations for 16 hours in 0 1N alkali but not after 24 hours incubation By the conventional tibial assay she found growth activity to be lost from the Wilhelmi growth hormone after 16 hours confirming our experience with PR 1 We have recently examined two samples of Wilhelmi growth hormone which Russell and Wilhelmi found to be free of nitrogen retaining power after 24 hours of alkali treatment and have observed that ketogenic activity is undiminished by this treatment (Table 2) Further studies are needed to establish with certainty the precise conditions necessary for this separation and to establish unequivocally that growth activity is completely lost

Table 2

EFFECT OF 24 HOUR EXPOSURE OF WILHELMI S GROWTH HORMONE TO 0 1N NaOH AT 25 C*

Sample		*Rise in Blood Ketones in 3 5 Hours Mg Per Cent as Acetone*	
		Untreated	*Alkali Treated*
B 169GH	1 mg	4 81 ± 0 61 (4)	6 31 ± 1 35 (5)
B 111 60GH	1 mg	6 87 ± 0 53 (4)	5 98 ± 0 68 (7)

* 5 mg of hormone was dissolved in 1 5 ml of 0 1N NaOH

It is of more than passing interest to remind the reader that some of the earliest studies on the growth and ketogenic activities of pituitary extracts suggested that alkali treatment might effect a separation of these two properties The original Burn and Ling [6] ketogenic extract was an alkaline

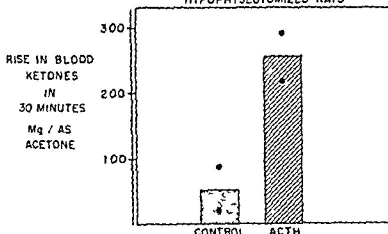


FIG. 4 Effects at 3½ hours of oxycel corticotropin on blood ketone rise in anesthetized hypophysectomized rats The study was performed three hours after operation

there is still profit in evaluating the existing experimental data on this subject

In Figure 5 are depicted in schematic form the intermediary metabolic pathways involved in ketosis and shown also are some of the possible mechanisms by which growth hormone (and/or the ketogenic factor) may initiate ketosis It will be recalled that the ketone bodies acetoacetate beta hydroxybutyrate and acetone are derived as acetoacetate from the liver during the metabolism of acetyl-coenzyme A fatty acids and certain amino acids Acetyl CoA is a meeting point for carboyhydrate protein and fat metabolism It may be formed during the catabolism of any one of these major fuels although its major source is probably from fatty acid catab olism and from pyruvate Once formed it may travel along a number of different metabolic pathways notable among which is its condensation with oxalacetate to enter the Krebs citric acid cycle Here it is completely oxidized and yields energy as ATP which is essential for many endogenous processes It can be traced on the main pathway in the conversion of carbohydrate to fat it enters into the synthesis of cholesterol and other steroids purines and porphyrins and it is involved in acetylation reactions Finally, and of particular interest to this discussion is its condensation with another molecule of acetyl CoA to form acetoacetyl CoA This latter com pound may also be formed as an intermediary during both the catabolism and anabolism of fatty acids It is formed in all tissues which are capable of burning fatty acids However it escapes into the blood stream from only one tissue the liver because of the unique existence in this organ of an

pituitary or adrenal cortex of the recipient In Figure 3 are shown data to the effect that oxycel ACTH induces ketosis in the hypophysectomized rat while in Figure 4 are presented data on the stimulation of ketosis by corticotropin in the adrenalectomized rat maintained with either desoxycorticosterone or cortisone acetate It should be noted that not only were considerably larger doses of corticotropin required than in intact rats but the response in ketosis was also less In view of the fact that even minor degrees of failure of the peripheral circulation depress fasting ketosis in the rat[8] the lesser ketosis in the anesthetized adrenalectomized or hypophysectomized rat need not be interpreted as indicating an intrinsic lack of responsivity to ketogenic factor in the absence of the pituitary or adrenal Indeed the unanesthetized adrenalectomized rat maintained in good condition with DOCA *develops greater than normal ketosis on fasting*[8] The response to corticotropin was significantly greater in the adrenalectomized rat maintained on 0 5 mg cortisone making it necessary to consider the possibility that the high ketogenic activity of corticotropin is based on a synergism between the ketogenic factor and adrenal steroids[36 37]

We come now to a consideration of the possible sites of action in intermediary metabolism of the ketogenic factor Although it is apparent from what has already been stated that a final solution to this problem must await a decision concerning the precise nature of the ketogenic factor

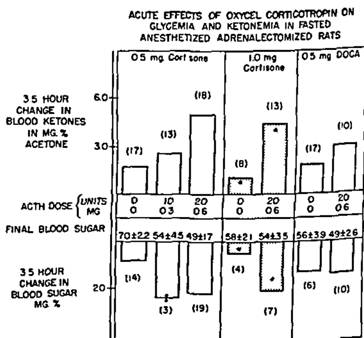


FIG 3 Effects at 3½ hours of oxycel corticotropin on blood ketone rise in adrenalectomized rats Data on the fall in blood sugar are also recorded

celerated simultaneously Under the influence of hepatic deacylase acetoacetate is released into the blood stream Ketone bodies tend to accumulate since the rate of release easily exceeds their rate of uptake and utilization by the extra hepatic tissue The continued oxidation of acetoacetyl CoA and acetyl CoA in muscle and other peripheral tissues is much less dependent on carbohydrate utilization than is the same process in liver since the latter contains an enzyme β decarboxylase which allows oxalacetate to drain out of the system Thus in the extra hepatic tissues a decline in oxalacetate does not readily occur and the oxidation of acetyl CoA may continue even when carbohydrate utilization is decreased It is well known that ketone utilization remains normal in diabetes mellitus It should be noted that CoA is involved in many steps in the metabolism of fat acetate and pyruvate Hence the continuing metabolism of these materials is dependent on the presence of CoA In this respect the formation of acetoacetate from acetyl CoA by the liver may be viewed as a means by which free CoA is returned to the liver when this process cannot be achieved at the usual rate, either by the complete synthesis of fat or by the oxidation of acetate in the Krebs cycle This enables fat catabolism to continue

In Figure 5 we have indicated by appropriate numbers those steps in metabolism which have been suggested as definite sites of action of growth hormone and/or adipokinin and the ketogenic factor The broken lines indicate slowing or inhibition and the unbroken lines indicate stimulation or speeding up of metabolic reactions under hormonal influence The following are the sites of action which have been demonstrated or strongly suggested by the experimental data They are indicated on the chart by the appropriate number some of the numbers are encircled and some are not depending on whether the action is an inhibiting or stimulating one respectively The dot beside the number should be overlooked

1 Inhibition of the hexokinase reaction [39]

2 Inhibition of a step in glycolysis below hexosediphosphate This is related to the R Q depressing and glycostatic activity of pituitary extracts [40]

3 Stimulation of the conversion of amino acids to protein and/or inhibition of amino acid catabolism [4, 41]

4 Mobilization of fat from the depots to the liver [4, 43, 44]

5 Acceleration of fatty acid catabolism [4]

6 Inhibition of the conversion of carbohydrate to fat or of the resynthesis of fat from acetyl CoA [46, 47] This might be considered as a consequence of 1 and 2

7 Inhibition of the oxidation of acetate via the Krebs cycle [4]

From the previous discussion and examination of Figure 5 it is clear that each of the seven suggested sites of action of the pituitary hormone(s) is such as to lead directly or indirectly to a stimulation of ketone body formation in the liver It is thus obvious that it will be very difficult to reach a final decision as to whether one or several pituitary hormones are

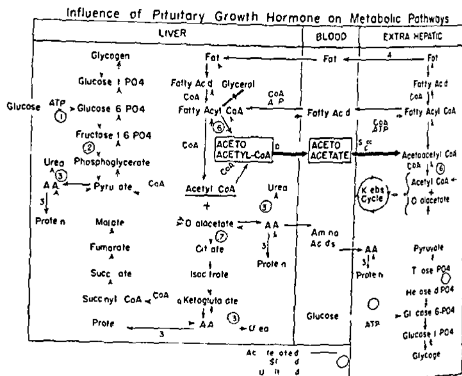


FIG 5 See text for description

enzyme deacylase which splits acetoacetyl CoA to acetoacetate and CoA All other tissues continue the metabolism of acetoacetyl CoA as indicated in Figure 5 Most tissues (notably heart skeletal muscle and kidney) possess an active enzyme system concerned with converting acetoacetate from the blood stream back to acetoacetyl CoA The CoA is derived from succinyl CoA during the oxidation of α ketoglutarate to succinate in the Krebs cycle Acetoacetyl CoA may then be used either catabolically via the Krebs cycle or it may enter into some of the synthetic reactions noted above

There are currently two prevailing views to explain ketosis within the framework of the metabolic pathways just outlined both views considering acetoacetate formation by the liver as an overflow pathway when for any reason there is a relative or absolute increase in acetyl CoA One relates an increased availability of acetyl CoA to a deficiency in oxalacetate (secondary to impaired carbohydrate utilization or to accelerated protein anabolism) with consequent slowing of the Krebs cycle The second view places major emphasis on a lowered rate of lipogenesis from carbohydrate and acetate which also would be a consequence of impaired utilization of glucose since fat synthesis is linked to some step in glycolysis [33] In either case the supply of acetyl CoA and acetoacetyl CoA would be considerably increased and this would be magnified if fatty acid catabolism were ac-

celerated simultaneously Under the influence of hepatic deacylase aceto acetate is released into the blood stream Ketone bodies tend to accumulate since the rate of release easily exceeds their rate of uptake and utilization by the extra hepatic tissue The continued oxidation of acetoacetyl CoA and acetyl CoA in muscle and other peripheral tissues is much less de pendent on carbohydrate utilization than is the same process in liver since the latter contains an enzyme, β decarboxylase which allows oxalacetate to drain out of the system Thus in the extra hepatic tissues a decline in oxalacetate does not readily occur and the oxidation of acetyl CoA may continue even when carbohydrate utilization is decreased It is well known that ketone utilization remains normal in diabetes mellitus It should be noted that CoA is involved in many steps in the metabolism of fat acetate and pyruvate Hence the continuing metabolism of these materials is de pendent on the presence of CoA In this respect the formation of acetoace tate from acetyl CoA by the liver may be viewed as a means by which free CoA is returned to the liver when this process cannot be achieved at the usual rate either by the complete synthesis of fat or by the oxidation of acetate in the Krebs cycle This enables fat catabolism to continue

In Figure 5 we have indicated by appropriate numbers those steps in metabolism which have been suggested as definite sites of action of growth hormone and/or adipokinin and the ketogenic factor The broken lines indicate slowing or inhibition and the unbroken lines indicate stimulation or speeding up of metabolic reactions under hormonal influence The fol lowing are the sites of action which have been demonstrated or strongly suggested by the experimental data They are indicated on the chart by the appropriate number some of the numbers are encircled and some are not depending on whether the action is an inhibiting or stimulating one re spectively The dot beside the number should be overlooked

1 Inhibition of the hexokinase reaction [39]

2 Inhibition of a step in glycolysis below hexosediphosphate This is related to the R Q depressing and glycostatic activity of pituitary extracts [40]

3 Stimulation of the conversion of amino acids to protein and/or inhibition of amino acid catabolism [24 41]

4 Mobilization of fat from the depots to the liver [42 43 44]

5 Acceleration of fatty acid catabolism [45]

6 Inhibition of the conversion of carbohydrate to fat or of the re synthesis of fat from acetyl CoA [46 47] This might be considered as a con sequence of 1 and 2

7 Inhibition of the oxidation of acetate via the Krebs cycle [45]

From the previous discussion and examination of Figure 5 it is clear that each of the seven suggested sites of action of the pituitary hormone(s) is such as to lead directly or indirectly to a stimulation of ketone body formation in the liver It is thus obvious that it will be very difficult to reach a final decision as to whether one or several pituitary hormones are

concerned with these various metabolic effects Since the chief purpose of this report is to consider the evidence for a direct influence of a pituitary hormone on fat metabolism and ketosis per se no further consideration will be given to those sites of action which produce secondary changes in fat and ketone metabolism (sites 1, 2 3 and 6) Nonetheless it should be kept in mind continually in the following discussion that these are operative and will potentiate any direct influences of the hormone on fat metabolism Wilhelmi[48, 49] has recently presented a very lucid resume of how fat catabolism and ketosis are modified indirectly by the effects of growth hormone on carbohydrate and protein metabolism

Any interpretation of the adipokinetic and ketogenic effects of pituitary extracts must take into account the great rapidity of these responses Liver fat accumulation is detectable within 30–60 minutes[17] while ketosis is well established in 30 minutes and hence, must begin immediately[1] (Fig 6) Greenbaum and McLean s demonstration[11] of an increase in plasma neutral fat concomitant with the rise in liver, neutral fat is strong evidence for a primary action of the hormone on fat mobilization from the periphery to the liver (site 4) At the present time little is known concerning the precise mechanism by which fat is released from the depots In view of the speed and magnitude of this response to the hormone it is difficult to conceive of it as being secondary to hormonal influences on carbohydrate and

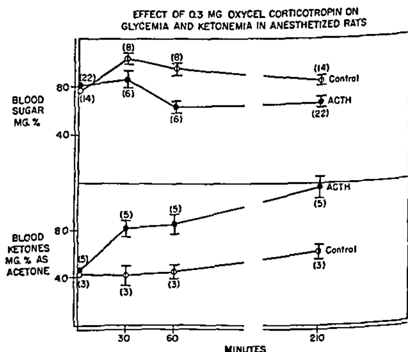


Fig 6 Effect of oxycel corticotropin on blood sugar and ketone levels in normal rats Note significant rise in blood ketones in 30 minutes

protein metabolism Since there is evidence that growth hormone treatment may also cause a secretion of insulin [10] which is known to have an opposite effect on fat mobilization an immediate direct effect of the hormone on fat mobilization best fits the experimental observations

The data of Young [9] and Greenbaum and McLean[41] indicate indirectly that fat mobilization and catabolism continue at an accelerated rate during prolonged administration of growth hormone until available fat depots are depleted Greenbaum and McLean consider this response as essential for the protein anabolic reaction to growth hormone and hence attribute to growth hormone a primary influence on fat metabolism Until the nature of the pituitary factor(s) involved in these reactions is established it is impossible to come to a decision as to whether the sustained effect on fat mobilization represents a primary or secondary response to one or more hormones

Equally difficult to analyze in these terms is the evidence for a direct influence of the hormone at site 5 since there are few data available to demonstrate an immediate effect on fatty acid catabolism per se or on the R Q although Greenbaum [1] did find a decline in the R Q within 24 hours As noted above the delayed effects are difficult to interpret Lotspeich and Petersen and Campbell and Davidson[53] found that liver slices from fasting rats exhibited increased oxygen consumption and acetoacetate pro duction within a few hours of treating the rats with pituitary extracts The latter investigators interpreted their findings as indicating an increased oxidation of endogenous fatty acids They could demonstrate no effect of the extract on oxygen consumption or ketone production when octanoate was added to the medium Greenbaum and McLean[45] found growth hormone to inhibit the fatty acid oxidase activity of liver homogenates dur ing the first six hours after treatment while after twenty four hours of treatment there was stimulation Octanoate and oleate were used as substrates and the system was so prepared that oxalacetate was not a limit ing factor This finding is difficult to reconcile with the known immediate increase in blood ketones after hormone treatment Since the total ketone production from octanoate and oleate was increased in the homogenates beginning 12–24 hours after growth hormone treatment of the rats and occurring without an increase in oxygen consumption Greenbaum and McLean suggest that the hormone may have a specific influence on the fate of acetate presumably in the Krebs cycle (site 7) Hunter [54] also found an increase in ketone production from octanoate in liver slices after 8 days of treatment with a preparation of Wilson s ACTH which we have shown to be rich in ketogenic activity [1] Thus so far the only evidence for a direct effect of pituitary hormone on oxidase activity of fatty acids has been after moderately prolonged treatment and the data of Greenbaum and McLean suggest that this might occur independently of an influence of the hormone on carbohydrate metabolism

We have attempted to derive further information on fatty acid catabolism by studying the influence of the ketogenic hormone on ketonemia in rats during the intravenous infusion of 1 5 per cent sodium octanoate So far, experiments have been continued up to 30 minutes after the injection of oxycel corticotropin The results are shown in Figure 7 During the 30-minute control saline infusion there was a small but significant increase in blood ketones which was taken as the baseline for the estimation of the influence of the different treatments At the end of the 30-minute infusion of 1 0 ml of 1 5 per cent sodium octanoate per 100 grams of body weight there was a further increase in blood ketones The saline infused rats which had received 0 120 mg of oxycel corticotropin 30 minutes previously, likewise showed a clear rise in ketone levels In the corticotropin treated rats receiving the octanoate, there was a considerable enhancement of ketosis which substantially exceeded that which would be anticipated from the sum of the octanoate and hormone effects as depicted in the last column of Figure 7 These results do not distinguish between a primary or secondary influence of the hormone on fatty acid catabolism and ketosis but do suggest that enhanced ketosis is not solely dependent on prior mobilization of neutral fat to liver Further studies of this type are planned

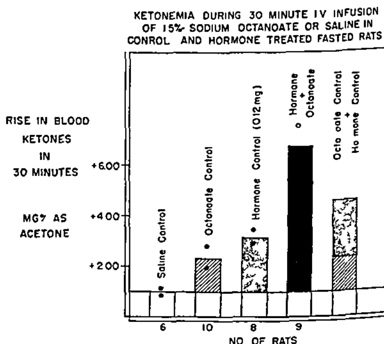


Fig 7 Rise in blood ketones in control and corticotropin treated rats during a 30 minute infusion of 1 0 ml of 1 5 per cent sodium octanoate per 100 grams body weight The chart is plotted from a baseline represented by the increase in blood ketones during infusion of saline alone The last column represents the sum of the octanoate and corticotropin effects corrected for the saline control

The isolated observations of Shipley and of Tepperman and Tepperman[36] that some samples of growth hormone may increase acetoacetate production when added directly to liver slices *in vitro* also is evidence that the ketogenic response is not dependent on prior mobilization of fat to the liver Indirectly these observations suggest that there is indeed an immediate influence of growth hormone on fatty acid catabolism but it must be recalled that in the liver slice this response could be secondary to an influence on carbohydrate metabolism In this regard Lotspeich and Petersen make much of the fact that they find a good correlation between the increase in acetoacetate production by the liver slice and the fall in blood sugar and liver glycogen two hours after injection of growth hormone Our own experience has been that there is no necessary correlation between the ketogenic and hypoglycemic activities of different pituitary preparations

Ketosis is a function of both hepatic ketone body production and peripheral utilization Although all our attention so far has been directed to the former it should not be assumed that a possible influence of the hormone on acetoacetate uptake and/or utilization can be completely ignored Bennett et al [6] and Mirsky [7] have performed studies on eviscerated animals and they interpreted the results as indicating no action of the pituitary factor on ketone body utilization Greenbaum suggested that this process might be impaired initially inasmuch as he observed a ketonemia without an increased production of acetoacetate by liver homogenates during the first 6 hours after hormone injection Since other investigators have found an early increase in ketone production by liver slices Greenbaum s suggestion is open to question Nevertheless this subject needs further investigation since a decrease in the uptake of acetoacetate by thick tissues would be a natural consequence of the overall metabolic pattern depicted in Figure 5 particularly if the amount of free CoA should become drastically reduced

This discussion has given no consideration to the possible role of other hormones in fat mobilization and ketosis It should be understood that this omission is one of temporary expediency with respect to time and space since it is obvious that insulin the adrenal cortex the thyroid and perhaps even glucagon are all involved to varying degrees Their respective contributions will have to be assessed in the final analysis of this problem

Summary

1 The ketogenic action of growth hormone has been discussed in terms of (a) the identity of the ketogenic factor and (b) the possible mode and sites of action of this factor in metabolism

2 Ketogenic activity has been found to variable degrees in different samples of corticotropin and growth hormone In general the most active preparations have been samples of oxycel purified corticotropin

3 The ketogenic action of oxycel corticotropin persists in hypophysecto-

We have attempted to derive further information on fatty acid catabolism by studying the influence of the ketogenic hormone on ketonemia in rats during the intravenous infusion of 1 5 per cent sodium octanoate So far experiments have been continued up to 30 minutes after the injection of oxycel corticotropin The results are shown in Figure 7 During the 30-minute control saline infusion there was a small but significant increase in blood ketones which was taken as the baseline for the estimation of the influence of the different treatments At the end of the 30 minute infusion of 1 0 ml of 1 5 per cent sodium octanoate per 100 grams of body weight there was a further increase in blood ketones The saline infused rats which had received 0 120 mg of oxycel corticotropin 30 minutes previously, likewise showed a clear rise in ketone levels In the corticotropin treated rats receiving the octanoate there was a considerable enhancement of ketosis which substantially exceeded that which would be anticipated from the sum of the octanoate and hormone effects as depicted in the last column of Figure 7 These results do not distinguish between a primary or secondary influence of the hormone on fatty acid catabolism and ketosis but do suggest that enhanced ketosis is not solely dependent on prior mobilization of neutral fat to liver Further studies of this type are planned

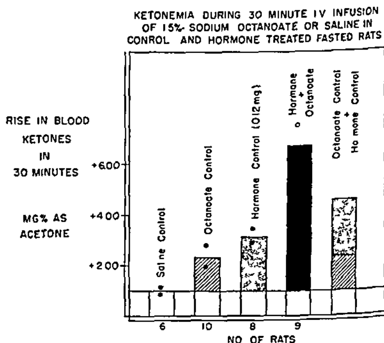


FIG 7 Rise in blood ketones in control and corticotropin treated rats during a 30 minute infusion of 1 0 ml of 1 5 per cent sodium octanoate per 100 grams body weight The chart is plotted from a baseline represented by the increase in blood ketones during infusion of saline alone The last column represents the sum of the octanoate and corticotropin effects corrected for the saline control

References

1 Engel F L and M G Engel *Endocrinology* (in press)
2 Scott J L Jr and F L Engel *Endocrinology* 53 410 (1953)
3 Engel M G and F L Engel *Endocrinology* (in press)
4 Raben M D and V W Westermeyer *Proc Soc Exp Biol Med* 78 550 (1951)
5 Greenspan F S Li C H Simpson M E and H M Evans *Endocrinology* 45 455 (1949)
6 Engel F L Viau A Coggins W and W S Lynn Jr *Endocrinology* 50 100 (1952)
7 Bogdonoff M D Scherr E H Owen J Lister L and F L Engel To be published
8 Myers J D Kibler R F Taylor W J Hamrick L M Engel F L and E E Werk *Int Physiol Cong Proc* XIX 638 (1953)
9 Engel F L Myers J D Werk E E Kibler R Bogdonoff M D Lister L Komrad E and M G Engel *Trans Assoc Am Physicians* (in press)
10 Bornstein J Reid E and F G Young *Nature* 168 903 (1951)
11 Foa P P Magid F B Glassman M D and H R Weinstein *Proc Soc Exp Biol Med* 83 758 (1953)
12 Werk E E McPherson H Hamrick L W Myers J D and F L Engel To be published
13 Lester D and L A Greenberg *J Biol Chem* 174 903 (1948)
14 Greenberg L A and D Lester *J Biol Chem* 154 177 (1944)
15 Michaels G D Margen S Siebert G and L W Kinsell *J Clin Invest* 30 1483 (1951)
16 Handler P *J Biol Chem* 173 295 (1948)
17 Rosenberg I N *Proc Soc Exp Biol Med* 82 701 (1953)
18 Reid E *J Endocrinology* 9 210 (1953)
19 Welt L D Personal communication
20 Astwood E B Raben M S Rosenberg I N and V W Westermeyer *Science* 118 567 (1953)
21 Westermeyer V W and M S Raben *Endocrinology* 54 173 (1954)
22 Astwood E B This volume
23 Russell J A Personal communication
24 Russell J A and M Capiello *Endocrinology* 44 333 (1949)
25 Russell J A This volume
26 Burn J H and H N Ling *Quart J Pharm and Pharmacol* 6 31 (1933)
27 Anselmino K J and F Hoffman *Klin Wschr* 10 2380 (1931)
28 Anselmino K J and F Hoffman *Klin Wschr* 13 1052 (1934)
29 Anselmino K J and F Hoffman *Endokrinologie* D 1 (1936)
30 Black P T Collip J B and D L Thomson *J Physiol* 82 385 (1934)
31 Shipley R A and C N H Long *Biochem J* (*London*) 32 2242 (1938)
32 Harrison H C and C N H Long *Endocrinology* 26 971 (1940)
33 Li C H and H Papkoff *J Biol Chem* 204 391 (1953)
34 Ellis S Noda G Simpson M E and H M Evans *J Biol Chem* 209 779 (1954)
35 Engel F L and K Hewson *Proc Soc Exp Biol Med* 83 608 (1953)
36 Tepperman J and H M Tepperman *Ann N Y Acad Sci* 54 707 (1951)
37 Petersen V B and W D Lotspeich *Federation Proc* 13 111 (1954)

mized and adrenalectomized rats although larger doses are necessary to elicit it than in the normal animal

4 A sample of Raben Westermeyer growth hormone has been tested and found to be active for growth diabetogenic and ketogenic activities in the rat for hyperglycemic activity in man and for adipokinetic activity in the mouse Incubation in 0 1N NaOH for 16–20 hours resulted in loss of growth, diabetogenic and hyperglycemic activities while ketogenic and adipokinetic activities remained Thirty minutes of boiling at pH 3.5 destroyed ketogenic and adipokinetic without reducing the other activities

5 A sample of Astwood oxycel corticotropin rich in ketogenic and adipokinetic activities retained these properties both after 16–20 hours of alkali treatment and after boiling at pH 3 5 The ketogenic activity of two samples of Wilhelmi growth hormone resisted 24 hours of exposure to 0 1N NaOH

6 The possibility was suggested that a ketogenic factor might be separated from growth hormone by these techniques

7 The mechanisms by which the pituitary factor might cause ketosis have been reviewed and the evidence presented to support the view that ketosis may result both from a direct influence on fat mobilization and catabolism and from an indirect consequence of the action of growth hormone in carbohydrate and protein metabolism

8 A ketogenic preparation of corticotropin increased the proportion of ketone bodies appearing in the blood during the intravenous infusion of 1 5% sodium octanoate This suggests that fat mobilization to the liver is not a prerequisite to ketosis and that the ketogenic hormone has a direct or indirect influence on fatty acid catabolism per se

Acknowledgments

I am much indebted to the following collaborators who have contributed to the experimental work recorded in this presentation and without whom this report would not be possible Mildred G Engel who carried out all studies on ketosis in the rat Drs M D Bogdonoff L Lister and J A Owen Jr who conducted the assays for diabetogenic activity of PR 1 in the rat, Dr H T McPherson who tested for adipokinetic activity in the mouse Drs E Werk Jr R Kibler and J D Myers who measured the hyperglycemic effect of PR 1 in man and Dr E Komrad who performed the growth assays and hypophysectomies

In addition I am indebted to Dr L Mitchell the Horner Company Ltd Montreal Dr David Klein the Wilson Laboratories Chicago Illinois Dr Irby Bunding the Armour Laboratories Chicago Dr A E Wilhelmi Emory University and Drs E B Astwood and M S Raben Boston Mass for generous supplies of pituitary hormones used in these studies

induced hyperketonemia and its inhibition with cortisone and related adrenal steroids We were perplexed on the other hand when he found in the rat that corticotropin preparations instead of inhibiting the fasting induced hyperketonemia caused an actual accentuation In the non diabetic human ACTH has the same suppressive effect upon blood ketones as does cortisone However in the diabetic human maintained on small amounts of insulin given adequate amounts of electrolytes and subjected to fast cortisone will again exert a suppressive effect upon hyperketonemia whereas ACTH or a substance which is highly purified corticotropin works in an opposite direction It would seem probable then that insulin in abundant amounts may be essential for this adipokinetic effect or for the ketone suppressing effect of the corticoids The adipokinetic or hyperketonemic effect is manifested in the diabetic receiving corticotropin or whatever it is that rides along with corticotropin because of insulin lack

I would like to make one comment in regard to Dr Russell s paper of this morning She found that a very high fat intake in the absence of carbohydrate resulted in a more obvious anabolic effect from administered growth hormone or from the pituitary material with growth activity than was achieved with a high carbohydrate intake After many failures the first study in which we were able to demonstrate a positive anabolic effect from such pituitary material given to human subjects was in a patient who was maintained on a formula diet containing no carbohydrate I mention this as a matter of interest but suggest however that this obviously cannot be an essential property of normal growth hormone inasmuch as people have been known to grow while they were consuming carbohydrate

PHILIP BONDY I wish to mention first of all that in our growth hormone experiments in humans we have observed the very early rise of blood ketone bodies which Dr Engel has found in rats This seems rather paradoxical in view of the findings Dr Greenbaum reported today in respect to his own experiments I may say that we also have found these changes under circumstances where there were no appreciable alterations in other chemical constituents of the blood bearing out the observations Dr Knobil reported this morning Recently we have been interested in the effects of growth hormone on fat synthesis and Dr Lipske in our laboratory has started studying the effects of this substance among others on the incorporation of labeled acetate (the C_{14} at the carboxyl carbon) by the plasma lipids of normal humans When I say normal I mean normal in respect to nutrition These patients all have cancer but they are as normal as a person can be with this disease We have found in two patients so studied a very appreciable diminution in the rate of incorporation of the label by both the total neutral fat and the phospholipid fractions This diminished incorporation was apparent within a very short time Fifty mg of a growth hormone preparation (Horner PR 1) was given intramuscularly 24 hours before and then as an

38 Chaikoff I L *Harvey Lectures Ser* **47** 99 (1953)
39 Colowick S P Cori G T and M W Slein *J Biol Chem* **168** 583 (1947)
40 Recant L *Federation Proc* **11** 273 (1952)
41 Hoberman H D *Yale J Biol and Med* **22** 341 (1950)
42 Szego C M and A White *Endocrinology* **44** 150 (1949)
43 Levin L and R K Farber *Recent Progr Hormone Research* **7** 399 (1952)
44 Greenbaum A L and P McLean *Biochem J* **54** 407 (1953)
45 Greenbaum A L and P McLean *Biochem J* **54** 413 (1953)
46 Welt L D and A E Wilhelmi *Yale J Biol and Med* **23** 99 (1950)
47 Gurin S and R O Brady *Recent Progr Hormone Research* **8** 571 (1953)
48 Wilhelmi A E *Ciba Foundation Colloquia on Endocrinology* **6** 70 (1953)
49 Wilhelmi A E *Experimental Diabetes A Symposium* Springfield Ill Charles C Thomas Publisher 1954 199
50 Young F G *Biochem J* **39** 515 (1945)
51 Greenbaum A L *Biochem J* **54** 400 (1953)
52 Lotspeich W D and V P Petersen *Am J Physiol* **176** 232 (1954)
53 Campbell J and I W F Davidson *J Biol Chem* **189** 35 (1951)
54 Hunter S F *Proc Soc Exp Biol Med* **82** 14 (1953)
55 Shipley R A *Am J Physiol* **141** 662 (1944)
56 Bennett L L Kreiss R E Li C H and H M Evans *Am J Physiol* **152** 210 (1948)
57 Mirsky A *Am J Physiol* **115** 424 (1936)

DISCUSSION

Growth Hormone and Energy Sources

General Discussion

CHARLES BEST (Chairman) Dr Tepperman was to have opened the discussion but he has been unable to attend Accordingly we will have questions or comments from individuals

ANNE MILMAN (New York Hospital) I might add a few words to the information on species differences with respect to the diabetogenic effect of growth hormone Perhaps Dr Bunding might like to know what became of some of the growth hormone he sent us We found that young growing chinchilla rabbits maintained on a constant food intake developed glycosuria when treated with growth hormone for a few days Glycosuria disappeared when growth hormone injections were stopped after a treatment period of about 3 weeks Growth hormone also increased nitrogen loss and diabetic signs in these young growing rabbits which had been treated with large doses of cortisone

LAURANCE KINSELL We were gratified some years ago when Dr Engel confirmed in the rat our findings in human subjects relating to the fasting

the point of actual anatomical destruction if one applies a heavy stimulus long enough

PAUL MARKS (National Institutes of Health) I have a question to ask Dr Greenbaum which concerns his experiments with 2 label pyruvate In view of the postulated action of growth hormone as possibly blocking glucose or fructose conversion to lactic acid has he made any observations on the incorporation of the label into glucose or glycogen?

JAMES CAMPBELL I was glad to hear some further discussion on the destructive effect of excessive amounts of growth hormone on the islets of Langerhans because I made the remark with this in mind Dr Lukens mentioned also that a serious decrease in the amount of kidney in an animal under certain circumstances can lead to a destruction of the remaining tissue And of course we should recall the work of Allen in partially depancreatized dogs in which he showed that a remnant of the pancreas can be overstrained to destruction

CHARLES BEST But that effect was not by other hormones

JAMES CAMPBELL That is correct

DAVID GREENBAUM There is only one question which was from Dr Marks and the answer is very simple I haven t a clue As I have pointed out the experiments are extremely preliminary and so far we have not reached the point of measuring anything except the fatty acids

FRANK ENGEL With respect to Dr Kinsell s comments I might point out in case there is any confusion that no basic contradiction exists between his observations of the ACTH inhibition of ketosis in man and ours of the stimulating action in the rat The difference of course is that he gave his ACTH as a prolonged intravenous infusion as I recall or possibly by using a gel I am not sure which one Under such circumstances however one would anticipate a good stimulation of the adrenal cortex with the secretion of its appropriate steroids We gave our material as a single large dose which in terms of ACTH units was quite tremendous even though by weight it was quite small We were seeing the immediate effect of the ketogenic factor stuck to or in some way associated with the ACTH

intravenous infusion about one hour before the label was given We considered that the patient was receiving a very large dose of hormone at the time of the experiment It is possible that the diminished labeling which followed was a result of the mobilization of lipid We doubt this for several reasons For one thing the fat mobilization would have had to double or more the plasma lipid concentration in order that dilution alone might be held accountable for the observed depression In the second place we saw no evidence of change in the plasma lipids, although we do not have enough data to confirm this completely

Finally one last point about a possible reason for the existence of growth hormone might be mentioned Dr F G Young some years ago made the ingenious suggestion that growth hormone might be necessary for the adjustments to fasting This concept has not been mentioned, so far, in the discussion except as a peripheral allusion by Dr Astwood We have been very much intrigued by this idea and it is disappointing to report that the pattern of incorporation of C_{14} carboxyl labeled acetate into the lipids of human subjects receiving growth hormone and glucose supplements in no way resembles the pattern seen in humans fasted for 24 hours Under the latter circumstances the rate of incorporation is diminished far below that observed following growth hormone That there is also a qualitative difference we cannot say presently The amounts of growth hormone used i e 300 mg intravenously over a period of 24 hours seem quite large and for the moment at least we can only say this doesn t seem to fit the pattern postulated by Dr Young

J C SHAW (University of Maryland College of Agriculture) This is a matter of species difference again I would just like to add that in the lactating cow we have a ketosis which is cured very rapidly by one injection of either ACTH or the adrenal steroids cortisone and hydrocortisone

KARL PASCHKIS I hope that all comments on the papers about fat metabolism are over because I would like to refer back to Dr Campbell s paper which has nothing to do with this subject Dr Campbell underscored the fact that destruction of or damage to the beta cells can be attributed partly at least to the effects of growth hormone It is interesting but more disturbing that a physiological agent a hormone leads to the injury or destruction of another gland Some years ago Dr Engel described destruction of the adrenal cortex through overstimulation with ACTH In our laboratories presently, there are experiments running which are concerned with the effects in guinea pigs of TSH given by constant infusion over a period of 96 hours The last experiment has been going for a somewhat longer period We have not seen to date any anatomical destruction but we have produced a real exhaustion state with a decrease of protein bound iodine and of I^{131} uptake One can overstimulate to the point of exhaustion and probably to

Part IV

Growth Hormone and Cellular Systems

Chairman

Carl F Cori
Washington University School of Medicine
Saint Louis Missouri

21

Effect of Pituitary Hormones on Metabolism of Isolated Tissues*

M E Krahl
Department of Physiology University of Chicago Chicago

Earlier papers of this symposium have reviewed experiments upon the relation of the anterior pituitary gland to the economy of the whole animal It is clear that fractions of the anterior pituitary which have high growth activity may produce effects on carbohydrate fat and nitrogen metabolism This paper is confined to experiments which have been made on isolated tissues to inquire into how the pituitary produces these effects in a general physiological or biochemical sense and into the chemical nature of the active substances It deals particularly with glucose utilization and glycogen synthesis Effects of pituitary products on glycogen breakdown have been reviewed by Russell[1] studies upon fat synthesis especially those of the Gurin and Chaikoff[3] groups have been summarized by Lukens[4] and Wilhelmi[5] recent experiments on fat synthesis are reviewed in this symposium by Folley and by Greenbaum No effects of pituitary products on amino acid deposition in isolated tissues have apparently yet been established

Pituitary Effects on Glucose Utilization by Tissues

In the human the dog the rat and presumably in other omnivores the presence of the anterior pituitary or its products at relatively low dosage serves to limit the conversion of glucose to CO thereby blood glucose is maintained to protect the brain against hypoglycemia Quantitatively the greatest use of glucose is in muscle adipose tissues and liver

I shall consider mainly experiments on isolated muscle These indicate first that glucose uptake or glycogen synthesis is inhibited by a pituitary

* This investigation was aided by grants from Eli Lilly and Company from the Wallace C and Clara A Abbott Memorial Fund of the University of Chicago and from the Life Insurance Medical Research Fund

and incubated in the desired medium glucose analyses are made before and after the incubation and the glucose uptake obtained by difference Our early experiments dealing with the relation of the anterior pituitary and the pancreas to muscle metabolism have been reviewed by my colleague Dr Park and myself[7 10 13] related findings by other investigators are also reviewed there

A HYPOPHYSECTOMY Krahl and Park[11] found that glucose uptake by diaphragms from rats hypophysectomized 10–20 days previously was 4 9 mg per gram per hour as compared to a control rate of 3 5 (Fig 1) This increase in glucose uptake at 10–20 days following hypophysectomy has been repeatedly observed by several members of our own group[9 1 13] by Villee and Hastings[14] and by Bornstein and Nelson[1] but not* by Li Kalman and Evans[16] The rise found after hypophysectomy is very much larger than that after adrenalectomy[11 17] diaphragms from rats lacking both the pituitary and adrenals have a rate of glucose uptake which is also high relative to normal[1 14] These findings are consistent with the fact that the proportion of carbohydrate used in the metabolism of the hypophysectomized animal is greater than that in the normal[13 18 0]

B INJECTION OF PITUITARY FRACTIONS

1 *Inhibition of glucose uptake* The factor from the pituitary which inhibits glucose uptake has been investigated with the diaphragm as test object (Fig 2) Crude alkaline extracts of the pituitary and once-crystallized growth hormone (3 mg per rat) produced 40–50 per cent depression of diaphragm glucose uptake when injected into hypophysectomized rats 3 hours prior to diaphragm removal[1] Inhibitory activity in this *3 hour test* was nearly lost when the growth hormone was recrystallized[13 1] the more highly purified samples exhibited relatively low activity in the 3 hour test even when adrenal cortical extracts or adrenocorticotropic fractions were concurrently administered The activities of various samples of growth hormone prepared by us according to the Wilhelmi method and of Armour 22KR2 kindly provided by Dr Bunding have been recorded by Park[10] Later it was found[1 13] that highly purified growth hormone was active in doses as small as 2 µg per rat if a longer time was allowed between injection and diaphragm removal (*24 hour test*) From these experiments it was concluded that the inhibitor of glucose uptake was not identical with growth hormone as currently obtained[7 10 23 24] but was some related substance which could perhaps be formed *in vivo* from or under the influence of growth hormone Further experiments bearing on the relation of growth fractions to the inhibitor are given below

* In view of the inhibition of hexokinase by glucose 6 phosphate[63] and the high concentration of this metabolite which may appear in stimulated muscle[6] it is possible that the effects of hormonal imbalance may be obscured by the inhibitory effects of the glucose 6 phosphate formed during diaphragm removal unless the diaphragms are subjected to a preliminary soaking period to extract excess glucose 6 phosphate

product and an adrenal cortical oxysteroid acting concurrently second that this pituitary product is related to but not identical with the growth hormone third that a pituitary dependent inhibitor of glucose uptake can be obtained in a lipoprotein fraction from the serum of diabetic animals and that a similar inhibitor is found in a lipoprotein fraction from the anterior pituitary itself Experimental observations bearing on these points will now be presented

I Isolated Muscle Excised rat diaphragms, when incubated in suitable media have been known for some time to use glucose and to respond to changes in glucose concentration and to insulin, in a manner similar to larger muscle aggregates [6 7 8] Being readily obtained in large numbers with reproducible properties and being thin and therefore, relatively accessible to sugars and other substances added to the incubation medium surrounding them diaphragms have been widely used to supplement more elaborate animal preparations in the study of physiologically active agents The procedure is simple although rather detailed precautions are necessary to obtain reproducible results [9] Diaphragms are removed from suitable donors

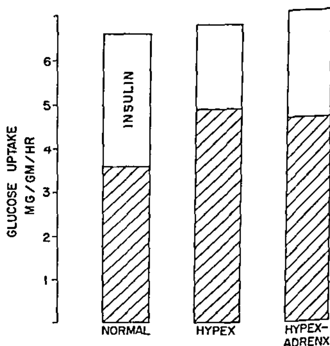


Fig 1 Glucose uptake by diaphragms from normal hypophysectomized or hypophysectomized adrenalectomized rats The excised muscle was shaken in Krebs bicarbonate solution and the medium analyzed for glucose before and after 30 minutes incubation at 37 C The initial glucose concentration of the medium was 140 mg per cent The height of the cross hatched area represents the rate without added insulin the total height represents the rate with 0 1 unit insulin per ml of incubation fluid The data are from Park and Daughaday [12]

mized adrenalectomized animals (Fig 1) Hence even when the rat has been deprived of both pituitary and adrenal secretions for some weeks the glucose uptake is limited by a factor in muscle whose effect is overcome by insulin

The inhibition of glucose uptake induced by limited single doses of growth hormone is almost completely insulin reversible (Figs 2 and 3)

From the experiments cited in Figures 1–3 it is concluded that the stimulating action of insulin upon glucose uptake is made up of two components the one is an action upon some factor in the tissue itself the other is upon a factor of pituitary adrenal origin The possible nature of the tissue factor will be considered below

Repeated doses of growth hormone over a 10 day period make it impossible for insulin to raise the rate of glucose uptake to the level obtainable without injection (Fig 2) Such an action of the pituitary factor upon the glucose uptake of muscle may well account in substantial degree for the insulin resistance which is observed with respect to other test indices

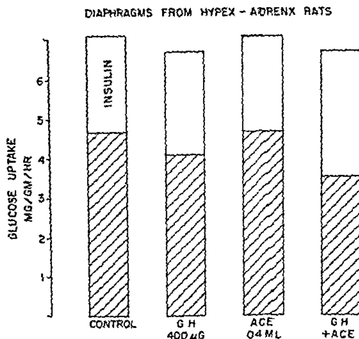


Fig 3 Glucose uptake by diaphragms from hypophysectomized adrenalectomized rats after injection of growth hormone (GH) and lipoadrenal extract Upjohn (ACE) The growth hormone was given 24 hours the lipoadrenal extract in divided doses 24 and 6 hours prior to diaphragm removal The diaphragms were incubated and the results expressed as described under Figure 1 The data are from Park Brown Cornblath Daughaday and Krahl[13]

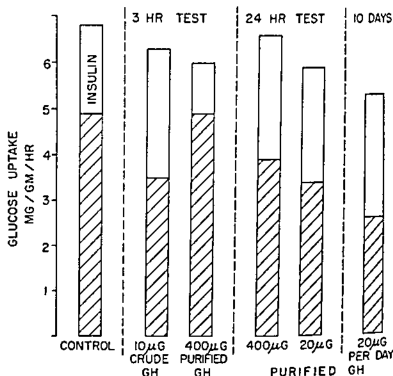


FIG 2 Glucose uptake by diaphragms from hypophysectomized rats given growth hormone (GH) prior to diaphragm removal The diaphragms were incubated and results expressed as described under Figure 1 For other details of the experiment see text The data are from Park Brown Cornblath Daughaday and Krahl [13]

For full inhibitory activity in the hypophysectomized adrenalectomized rat a trace of adrenal cortical extract was required (Fig 3) Injection of the pituitary growth fraction alone gave a slight decrease in glucose uptake by the diaphragm the same dose given concurrently with a small dose of adrenal cortical extract itself without effect was strongly active An analogous synergistic or permissive [5] action of adrenal steroids in relation to the metabolic effects of growth fractions has been observed with respect to the anti insulin effects on glycogen synthesis in diaphragms [8] the increase in insulin tolerance in dogs[26] and the production of metahypophyseal diabetes in cats [27]

2 *Relation to action of insulin* The experiments of Figures 1–3 with pituitary products have a bearing on the action of another hormone insulin Diaphragms from hypophysectomized adrenalectomized rats like the diaphragm donor animals themselves are still responsive to insulin the glucose uptake *with insulin* present in the incubation medium is about the same for diaphragms from normal from hypophysectomized and from hypophysecto

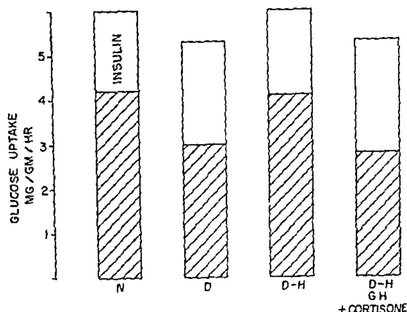


FIG 4 Glucose uptake by normal diaphragms in sera from normal (N) rats diabetic (D) rats diabetic hypophysectomized (DH) rats and diabetic hypophysectomized rats injected with growth hormone and cortisone in the latter case doses of 0 2 mg growth hormone and 0 1 mg cortisone were given intraperitoneally at 48 hours and again at 24 hours prior to diaphragm removal The diaphragms were incubated and the results expressed as described under Figure 1 The data are from Bornstein and Park [31]

2 *Inhibition by lipoprotein fractions* The inhibitor in sera was found to be destroyed by freezing suggesting that it might be a large and labile entity On the basis of this observation and my previous suggestion that lipoproteins should be put to use as solubilizing agents for adrenal steroids Bornstein tested the effect of a lipoprotein fraction from diabetic rat plasma upon glucose uptake by normal diaphragm [4] he found it to be inhibitory (Fig 5) He reported that other fractions from the same diabetic plasma were without inhibitory effect

Bornstein and I encouraged by these findings tested effects of lipoprotein fractions from diabetic rat plasma and from beef anterior pituitaries upon glucose utilization in cell free muscle extracts fortified with ATP and other components of the glucokinase system [3] strong inhibition was obtained (Fig 6) It will be remembered that Colowick Cori and Slein[36] obtained inhibition in such a test system with a certain proportion of the crude pituitary extracts tried although not with a sample of purified growth hormone supplied by Dr Li the inhibitor was highly unstable Results

the glycogen synthesis in diaphragms from rats given large doses of growth hormone,[8] the blood sugar of the dog given repeated doses of growth hormone [8] and the blood sugar of the human subject with active acromegaly

3 *Other effects* In addition to the inhibitory effects of growth fractions upon glucose uptake which are consistent with and help to explain the long term inhibitory effects of growth fractions on carbohydrate utilization in whole animals, there are transient effects in the opposite direction A small increase in glucose uptake by diaphragms[13 9] and hypoglycemia in whole or eviscerated animals [13 8] Park and co workers[13] were unable to separate the factors responsible for this effect from the growth activity but Westermeyer and Raben[30] have reported tests with a fraction having a high ratio of hypoglycemic to growth activity Earlier observations upon this interesting effect have been summarized by Park [10]

C INHIBITION OF GLUCOSE UPTAKE BY PLASMA AND BY LIPOPROTEIN FRACTIONS ADDED TO DIAPHRAGMS IN VITRO

I should now like to turn to a number of experiments which although too recent to have been subjected to adequate independent repetition seem to me to presage a substantial advance in our understanding of the metabolic effects of pituitary products They represent the first instance in which it has been possible to obtain a consistent inhibition of glucose uptake of muscle by addition of a factor of pituitary origin directly to the incubation medium *in vitro*

1 *Inhibitor in diabetic plasma* Upon the hypothesis that the inhibitory effects following injection of pituitary fractions were mediated by a hormonal factor Bornstein and Park[31] measured the effects of sera from animals with hormonal imbalance upon glucose uptake by normal diaphragms Tuerkischer and Wertheimer[3] had previously found glycogen synthesis by normal diaphragms to be subnormal in diabetic sera

The rates of glucose uptake by normal diaphragms in various types of sera were found to be as follows (Fig 4) in normal sera 4 2 mg per gram per hour in diabetic sera 3 0 in sera from diabetic hypophysectomized rats 4 1 in sera from diabetic hypophysectomized rats injected with growth hormone and cortisone 2 8 The presence of the inhibitor in sera from diabetic hypophysectomized rats was dependent on injection of *both* growth fraction and cortisone into the serum donor *neither agent would give the effect if injected singly neither could contribute to the effect if merely added to the medium* These findings extend those from the three hour injection test mentioned above in indicating that growth hormone as currently prepared is related to the factor which inhibits glucose uptake of muscle, but not identical with it The question of the full identity of growth hormone with the pituitary factor which produces metahypophyseal diabetes has also recently been raised [33]

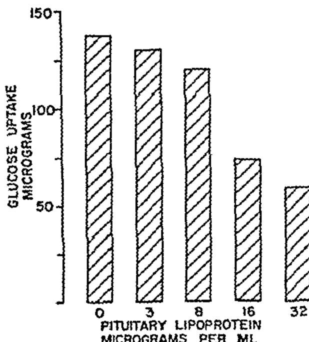


FIG 6 Effect of a lipoprotein fraction from the anterior pituitary on glucose uptake (disappearance) in a cell free muscle extract fortified with ATP and other components of the glucokinase system The data are from Krahl and Bornstein [35]

been reported by the DeDuve group[42] that insulin favors the synthesis of glycogen from glucose but not from fructose in normal liver slices

Indirect evidence for an inhibitor of glucose utilization in liver was found by Bornstein [34] It had previously been demonstrated[43] that in slices from livers with low glycogen content glutathione synthesis was dependent on the availability of glucose and its metabolites from the medium In Bornstein s experiments (Fig 7) normal liver slices from fasted rats (low liver glycogen) or fed rats (high liver glycogen) were incubated in a medium containing 200 mg per cent glucose Lipoprotein from diabetic rat plasma inhibited glutathione synthesis in livers from *fasted* rats but not that in livers from *fed* rats The difference indicates that the inhibitor acts on a step in glucose utilization which is by passed when glycogen is the source of energy yielding intermediates for synthesis of glutathione according to current views this is the step in which glucose is converted to glucose 6 phosphate This effect of diabetic serum lipoprotein on liver is in part reversed by insulin

III **Isolated Pancreas** Evidence for direct inhibition of glucose utilization in pancreas by a pituitary growth fraction was obtained by Anderson and Long [44] They found that insulin output by the perfused pancreas increased

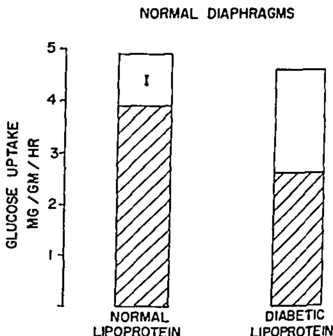


FIG 5 Glucose uptake by normal diaphragms in media containing lipoprotein from normal or diabetic rats The diaphragms were incubated and the results expressed as described under Figure 1 The data are from Bornstein [34]

related to those of Colowick et al have been obtained by others [37 40] In view of the previous difficulties I wish to emphasize that no sample of pituitary lipoprotein prepared as described [35] has failed to give an inhibition that the quantity required is relatively small (of the order of 3–30 μg per ml) and that the inhibitory activity is retained for some time under suitable storage conditions The recognition of the inhibitory action of pituitary lipoprotein fractions therefore represents a promising practical advance Because of the well known difficulties in ascribing biological activity to a particular fraction of the anterior pituitary these results must be viewed with some reserve pending further experimental work

II **Liver Slices** The glucose uptake by liver slices from hormonally deficient rats has been calculated by Renold Teng Nesbett and Hastings [41] Values for glucose uptake as μM per g wet liver in 90 minutes were found to be as follows for liver slices from various groups of animals normal 61 alloxan diabetic 29 diabetic adrenalectomized 24 diabetic hypophysectomized 44, adrenalectomized 53 hypophysectomized 49 This evidence would be consistent with an inhibitor of pituitary origin The situation is somewhat different than in the case of the diaphragm where either adrenalectomy or hypophysectomy of diabetic rats restores glucose uptake of the diaphragm to the normal level or above [14 17 31] In related experiments it has

mation of CO fat [3] and peptides[43] in these tissues the unused glucose appearing as high blood sugar Reduced glucose uptake by pancreatic islets might lead to decreased insulin synthesis[44] and eventually because of insufficient energy supply for maintenance to islet-cell breakdown and metahypophyseal diabetes Reduced glucose uptake in cells of adipose tissue might prevent optimum fat storage or lead to actual cell breakdown with discharge of fat Thus through the single agency of an inhibition of glucose uptake a number of the principal actions of the pituitary upon major metabolic components could possibly be explained

The second line of speculation concerns the importance of lipoproteins as carriers of biological regulatory activity The fact that the well characterized substances obtained from the pituitary are virtually devoid of activity when added to incubation media for individual tissues while lipoproteins from the pituitary are apparently intensely inhibitory suggests that such lipoproteins may have wide regulatory function Lipoproteins are intimately concerned with the structure and permeability properties of whole cells [48] the structure of cell formed elements[49,50] and the activity of individual enzyme systems [51,5] Lipoproteins of blood are carriers of physiologically active steroids [53,54] The possibility thus arises that a number of hormonal agents may interact with cellular or hormonal lipoproteins to produce changes in metabolic activity In particular it is suggested that interactions of insulin with lipoproteins may underlie both the changes in tissue distribution of sugars[55,57] and the changes in enzymatic activity[35,40,58] which have been reported to occur under the influence of this hormone Experiments to test this hypothesis are under way

The third line of speculation is concerned specifically with the mechanism by which growth fractions favor nitrogen retention and protein synthesis It is suggested that such a pituitary induced rearrangement of cell structure as that postulated above which leads directly or indirectly to a limitation in the transfer of high energy phosphate to glucose may divert into peptide bond synthesis an optimal fraction of the energy available for intracellular activities It has previously been pointed out by Lee and Ayers[59] and by Best[60] that movement of fat from depots under the influence of the pituitary growth factor tends to divert energy into growth

Summary

This review summarizes the effects of pituitary products on tissues Rat diaphragm muscle has been used as the principal test object The main findings are as follows

1 Glucose uptake by rat diaphragm *in vitro* is enhanced by hypophysectomy of the rat 10–20 days prior to removal of the diaphragm (Fig 1)

2 Glucose uptake by diaphragms from hypophysectomized rats is reduced toward normal by injection into the rats of crude growth hormone 3 hours before diaphragm removal (Fig 2) To achieve the same result

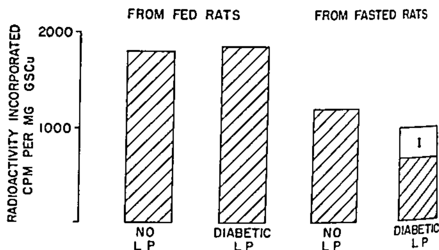


Fig 7 Effect of diabetic lipoprotein on incorporation of glycine 1-C^{14} into glutathione by liver slices from fed or fasted normal rats For details of the experiment see text The data are from Bornstein [34]

as the glucose content of the perfusate was increased being negligible at 35–84 mg per cent glucose and significant at 141–569 mg per cent This insulin secretion in response to elevation of the glucose concentration was blocked by inclusion of about 0 5 mg growth hormone per 100 ml perfusate Only one sample of growth hormone is mentioned it is not known whether this effect is obtainable with some samples of growth hormone but not others as is the case with the inhibitory effect of growth fractions on glycogen breakdown in rat diaphragm [4]

General Discussion

From the observations described above there arise three lines of speculation upon which I should like to comment

The first concerns the general importance of glucose uptake with respect to tissue function There is direct evidence that some factor from the pituitary related to growth hormone may inhibit glucose uptake by diaphragm muscle there is less direct evidence that such a factor may be able to reduce glucose utilization by liver and pancreatic islets It is of interest to consider briefly the possible physiological consequences of inhibition of glucose uptake in various tissues by such an agent it is assumed in each instance that the tissue in question can function optimally only with an adequate carbohydrate supply as is apparently the case for liver [43 46 47] Reduced glucose uptake by muscle and liver might lead to decreased for

mation of CO fat [3] and peptides[43] in these tissues the unused glucose appearing as high blood sugar Reduced glucose uptake by pancreatic islets might lead to decreased insulin synthesis[44] and eventually because of insufficient energy supply for maintenance to islet-cell breakdown and metahypophyseal diabetes Reduced glucose uptake in cells of adipose tissue might prevent optimum fat storage or lead to actual cell breakdown with discharge of fat Thus through the single agency of an inhibition of glucose uptake a number of the principal actions of the pituitary upon major metabolic components could possibly be explained

The second line of speculation concerns the importance of lipoproteins as carriers of biological regulatory activity The fact that the well character ized substances obtained from the pituitary are virtually devoid of activity when added to incubation media for individual tissues while lipoproteins from the pituitary are apparently intensely inhibitory suggests that such lipoproteins may have wide regulatory function Lipoproteins are intimately concerned with the structure and permeability properties of whole cells [48] the structure of cell formed elements[49,50] and the activity of individual enzyme systems [51,5] Lipoproteins of blood are carriers of physiologically active steroids [53,54] The possibility thus arises that a number of hormonal agents may interact with cellular or hormonal lipoproteins to produce changes in metabolic activity In particular it is suggested that interactions of insulin with lipoproteins may underlie both the changes in tissue distribution of sugars[55,57] and the changes in enzymatic activity[3, 40,58] which have been reported to occur under the influence of this hormone Experiments to test this hypothesis are under way

The third line of speculation is concerned specifically with the mechanism by which growth fractions favor nitrogen retention and protein synthesis It is suggested that such a pituitary induced rearrangement of cell structure as that postulated above which leads directly or indirectly to a limitation in the transfer of high energy phosphate to glucose may divert into peptide bond synthesis an optimal fraction of the energy available for intracellular activities It has previously been pointed out by Lee and Ayers[59] and by Best[60] that movement of fat from depots under the influence of the pituitary growth factor tends to divert energy into growth

Summary

This review summarizes the effects of pituitary products on tissues Rat diaphragm muscle has been used as the principal test object The main findings are as follows

1 Glucose uptake by rat diaphragm *in vitro* is enhanced by hypophysec tomy of the rat 10–20 days prior to removal of the diaphragm (Fig 1)

2 Glucose uptake by diaphragms from hypophysectomized rats is reduced toward normal by injection into the rats of crude growth hormone 3 hours before diaphragm removal (Fig 2) To achieve the same result

with purified growth hormone 24 hours are required From these and related experiments it is concluded that the active inhibitor is related to growth hormone but not identical with it

3 A factor from the adrenal cortex must be present for the full inhibitory activity of injected growth hormone to be manifested in hypophysectomized adrenalectomized rats (Fig 3)

4 A pituitary dependent factor which inhibits glucose uptake by diaphragms *when added directly to the incubation medium* has been found in diabetic serum (Fig 4) This is not demonstrable in the serum of hypophysectomized diabetic animals It reappears in the serum of the diabetic hypophysectomized rat after injection of growth hormone and cortisone prior to removal of the blood for serum preparation *both must be injected* the inhibition is not restored by injection of one and addition of the other to the serum *in vitro* or by addition of both to the serum

5 The inhibitor which acts directly on glucose uptake by diaphragms *in vitro* has been found in the lipoprotein fraction of diabetic serum or plasma (Fig 5)

6 A lipoprotein from the anterior pituitary inhibits glucose uptake when added directly to cell free muscle extracts fortified with the necessary components of the glucokinase system (Fig 6)

7 As a speculation it is suggested that a number of the effects of pituitary extracts may arise from inhibition of glucose uptake in the tissues concerned for example reduced glucose uptake may lead in liver and muscle to reduced CO glycogen fat and protein formation the unused glucose appearing as high blood sugar, in adipose tissue to discharge of stored fat and in pancreatic islets to reduced insulin formation and meta hypophyseal diabetes

8 As a further speculation it is pointed out that an effect of insulin to alter the properties of cellular and hormonal lipoproteins may account for the change in tissue distribution of sugars and the increased glucose uptake which insulin has been reported to produce

9 A mechanism for the favorable effect of growth hormone upon nitrogen retention is suggested

References

1 Russell J A *Ciba Foundation Colloquia on Endocrinology* Ed G E W Wolstenholme Boston Little Brown & Company VI 193 (1953)

2 Brady R O Lukens F D W and S Gurin *J Biol Chem* 193 459 (1951)

3 Chaikoff I L *Harvey Lectures 1951–52* 47 99 (1953)

4 Lukens F D W *Ciba Foundation Colloquia on Endocrinology* Ed G E W Wolstenholme Boston Little Brown & Company VI 55 (1953)

5 Wilhelmi A E *Ciba Foundation Colloquia on Endocrinology* Ed G E W Wolstenholme Boston Little Brown & Company VI 70 (1953)

6 Gemmill C L *Bull Johns Hopkins Hospital* **68** 329 (1941)
7 Krahl M E *Ann N Y Acad Sci* **54** 649 (1951)
8 Stadie W C *Physiol Rev* **34** 52 (1953)
9 Brown D H Park C R Daughaday W H and M Cornblath *J Biol Chem* **197** 167 (1952)
10 Park C R *Phosphorus Metabolism* Eds McElroy W D and B Glass Baltimore Johns Hopkins Press **II** 634 (1952)
11 Krahl M E and C R Park *J Biol Chem* **174** 939 (1948)
12 Park C R and W H Daughaday *Federation Proc* **9** 212 (1950)
13 Park C R Brown D H Cornblath M Daughaday W H and M E Krahl *J Biol Chem* **197** 151 (1952)
14 Villee C A and A B Hastings *J Biol Chem* **179** 673 (1949)
15 Bornstein J and J F Nelson *Nature* **162** 572 (1948)
16 Li C H Kalman C and H M Evans *Arch Biochem* **22** 357 (1949)
17 Krahl M E and C F Cori *J Biol Chem* **170** 607 (1947)
18 Drury D R *Am J Physiol* **111** 289 (1935)
19 Fisher R E and R I Pencharz *Proc Soc Exp Biol Med* **34** 106 (1936)
20 Shorr E *Cold Spring Harbor Symposia Quant Biol* **7** 323 (1939)
21 Park C R and M E Krahl *J Biol Chem* **181** 247 (1949)
22 Wilhelmi A E Fishman J B and J A Russell *J Biol Chem* **176** 735 (1948)
23 Krahl M E *The Diabetic Journal* (London) **6** 52 (1951)
24 Krahl M E *Diabetes* **2** 26 (1953)
25 Ingle D *Acta Endocrinologica* (in press)
26 Sinkoff M W de Bodo R C Den H and S P Kiang *Am J Physiol* **176** 361 (1954)
27 Lockett M F Reid E and F G Young *J Physiol* **121** 28 (1953)
28 de Bodo R C and M W Sinkoff *Recent Progress in Hormone Research* **8** 511 (1953)
29 Ottaway J H *Nature* **167** 1064 (1951)
30 Westermeyer V W and M S Raben *Endocrinology* **54** 173 (1954)
31 Bornstein J and C R Park *J Biol Chem* **205** 503 (1953)
32 Tuerkischer E and E Wertheimer *Biochem J* **42** 603 (1948)
33 Reid E *J Endocrinology* **9** 322 (1953)
34 Bornstein J *J Biol Chem* **205** 513 (1953)
35 Krahl M E and J Bornstein *Nature* **173** 949 (1954)
36 Colowick S P Cori G T and M W Slein *J Biol Chem* **168** 583 (1947)
37 Reid E Smith R H and F G Young *Biochem J* **42** XIX (1948)
38 Broh Kahn R H and I A Mirsky *Science* **106** 148 (1947)
39 Weil Malherbe H *Nature* **165** 155 (1950)
40 Abood L G and R W Gerard *Proc Soc Exp Biol Med* **77** 438 (1951)
41 Renold A E Teng C T Nesbett F B and A B Hastings *J Biol Chem* **204** 533 (1953)
42 Berthet J Jacques P Hennemanne G and C DeDuve *Arch intern physiol* **42** 282 (1954)
43 Krahl M E *J Biol Chem* **200** 99 (1953)
44 Anderson E and J A Long *Endocrinology* **40** 98 (1947)
45 Recant L *J Clin Invest* **31** 656 (1952)
46 Baker M Chaikoff I L and A Schusdek *J Biol Chem* **194** 435 (1952)

47 Masri M S Lyon I and I L Chaikoff *J Biol Chem* **197** 621 (195?)
48 Parpart A K and R Ballentine *Trends in Physiology and Biochemistry* Ed E S G Barron New York Academic Press Inc 1952 135
49 Claude A *Cold Spring Harbor Symposia Quant Biol* **9** 263 (1941)
50 Lazarow A *Biol Symposia* **10** 9 (1943)
51 Ball E G and O Cooper *J Biol Chem* **180** 113 (1949)
52 Braganca B M and J H Quastel *Biochem J* **53** 88 (1953)
53 Roberts S and C M Szego *Endocrinology* **39** 183 (1946)
54 Oncley J L Gurd F R N and M Melin *J Am Chem Soc* **72** 458 (1950)
55 Goldstein M S Henry W L Huddlestun B and R Levine *Am J Physiol* **173** 207 (1953)
56 Park C R *J Clin Invest* **32** 593 (1953)
57 Drury D R and A N Wick *Am J Physiol* **171** 721 (1952)
58 Krebs H A and L V Eggleston *Biochem J* **32** 913 (1938)
59 Lee M and G B Ayres *Endocrinology* **20** 489 (1936)
60 Best C H Personal communication
61 Weil Malherbe H and A D Bone *Biochem J* **49** 339 (1951)
62 Crane R K and A Sols *J Biol Chem* **203** 273 (1953)
63 Krahl M E Keltch A K Walters C P and G H A Clowes *J Gen Physiol* **38** 31 (1954)
64 Cori G T and C F Cori *J Biol Chem* **99** 493 (1933)

22

Effect of Growth Hormone on Transaminases and Other Enzyme Systems*

Oliver H Gaebler
Edsel B Ford Institute for Medical Research Henry Ford Hospital Detroit

Changes in the activity of tissue enzymes which occur after hypophysectomy or during treatment of normal or hypophysectomized animals with growth hormone are numerous and intriguing An endless amount of time could be devoted to purely descriptive studies in this area It is far more difficult to make any substantial progress in evaluating the significance of the observed changes Are they essential prerequisites for growth or merely interesting concomitants of the process? Does presence or absence of the pituitary influence growth by altering the activity of tissue enzymes or do changes in tissue enzyme activity occur secondarily after growth has been accelerated or stopped in some other manner? Are the findings related to the function of enzymes as catalysts or to their nature as protein components of tissue?

Although final answers to all of these questions may be long delayed there are experimental approaches which yield information concerning them In one of the early studies in this field Fraenkel Conrat Simpson and Evans[1] varied the dose of the anterior pituitary preparations In hypophysectomized rats growth hormone decreased hepatic arginase only when the dose was about ten times as great as that required to produce marked gain in weight Varying the method of feeding is also instructive We have evidence that some tissue enzymes accumulate in hypophysectomized rats and in normal ones pair fed against them apparently because growth is arrested by the operation in the former instance and by limited food intake in the latter Conversely Levin found that losses of total protein and water occurring in hypophysectomized adult rats can be prevented by forced feed

* Aided by a grant from the Michigan Chapter Arthritis and Rheumatism Foundation

ing Calculating results by several different methods likewise yields information of value Activity of an enzyme per gram of fresh tissue and per milligram of nitrogen may decrease, while the amount per organ increases In other words the enzyme appears to be synthesized more slowly than other components of the organ A fourth approach is that of comparing tissue enzyme activities during normal growth and induced growth Decreases in enzyme activity may occur in treated hypophysectomized rats that grow rapidly but not in normal rats that grow still faster

Studies carried out in our laboratory included assays of eleven tissue enzymes in one to four organs or tissues of normal and hypophysectomized rats, treated or untreated with growth hormone Conclusions based on the assumption that changes in apoenzyme concentration produced the observed changes in activity have been summarized previously[3] This morning I wish to discuss experiments in which certain enzymes were selected for detailed study on the basis of two criteria First the enzymes in question could logically be involved in such processes as skeletal development or nitrogen storage which are influenced by growth hormone, second the activity of the enzymes changed in the direction required to facilitate these processes In this discussion activity is of primary importance and points where proportionality between it and apoenzyme concentration is assumed are readily recognized

Let us first consider alkaline phosphatase of bone The views of Kay[4] and of Robison[5] concerning the role of this enzyme in bone formation are well known In 1947, Buchwald and Hudson[6] reported that its activity in bone fell after hypophysectomy Mathies and I confirmed this, and found that growth hormone reversed the change[7] The preparation which we used when assayed by the method of Marx Simpson and Evans[8] was only 40 per cent as potent as the best available ones and a large dose—1 mg per rat per day for 20 days—was used Mathies, Goodman and Palm[9] found that 50 micrograms of thyroxen injected daily for 7 days restored tibial alkaline phosphatase activity to normal in hypophysectomized rats without causing any increase in weight of the tibia Realizing that contamination with thyrotropic hormone might therefore be quite important they repeated and confirmed the previous experiments using a growth hormone preparation of sufficient purity to be highly effective when injected in a dose of 20 gamma per day for 10 days Experiments in which magnesium was added to the substrate indicated that deficiency of this ion could not account for the low alkaline phosphatase activity of tibia after hypophysectomy but might be one of the factors involved

In the same series of experiments[9] it was found that the effect of growth hormone on alkaline phosphatase of tibia persists when treatment is begun six and one half months after hypophysectomy Li Kalman Evans and Simpson[10] reported that a 2 week delay after hypophysectomy diminished the effectiveness of growth hormone in producing gain in weight In the

same laboratory[11] the osteogenic effect was demonstrated a year after the operation It is of interest that the effect on alkaline phosphatase activity like that on osteogenesis is a persistent one The response to growth hormone provides another instance of parallelism between alkaline phosphatase activity of bone and bone formation the response to thyroxin provides another exception The latter hormone in the dose employed restores alkaline phosphatase activity in the tibia of hypophysectomized rats but no gain in weight of the bone occurs

Mathies and coworkers[9] were able to show also that the decrease in tibial alkaline phosphatase which occurs after hypophysectomy is definitely secondary to cessation of growth The enzyme was determined in two groups of rats one of which was sham operated the other hypophysectomized In both groups alkaline phosphatase activity of the tibia decreased with age In the hypophysectomized group this activity was below that of the controls throughout an 80-day period of observation following the operation Growth of the tibia became minimal at once after hypophysectomy yet for three weeks alkaline phosphatase remained above values observed later in sham operated animals whose tibiae continued to grow Arrest of growth of the bone is clearly not due to lack of this enzyme One would scarcely expect such a relationship since alkaline phosphatase has been identified primarily with calcification rather than with osteogenesis in general

Other studies that I wish to discuss are related to the problem of nitrogen storage Reference has been made to the interest of earlier investigators[1] in effects of growth hormone on hepatic arginase which is involved in the process of urea formation Effects of another anabolic hormone testosterone

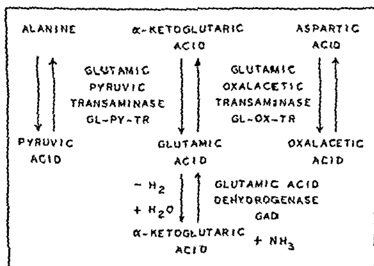


Fig 1

propionate upon this enzyme in liver and kidney have been studied extensively by Kochakian [12] Our own studies have been concerned with a group of enzymes which according to the theory of Braunstein and coworkers [13] provide an important mechanism for amino acid catabolism Alternatively the same enzymes are involved in synthesis of amino acids from alpha keto acids The names of the enzymes, the equilibria which they catalyze and the abbreviations which appear in later figures, are shown in Figure 1 Glutamic pyruvic transaminase and glutamic oxalacetic transaminase catalyze the equilibria represented in the upper part of this chart The amino group of alanine in one instance and of aspartic acid in the other, is transferred to alpha ketoglutaric acid which is thus converted to glutamic acid The latter is oxidatively deaminated by glutamic acid dehydrogenase If the ammonia is removed and ultimately converted to urea, these reactions constitute a pathway of amino acid catabolism

Studies concerning the effect of growth hormone on the activity of glutamic oxalacetic transaminase in muscle liver and kidney were begun in our laboratory by Bartlett and Glynn [14] Some of their findings are presented in Figure 2 The first four groups of animals were hypophysectomized Groups 2 3 and 4 received the indicated amounts of growth hormone in the course of 10 days Glutamic oxalacetic transaminase activity was high in the muscle of untreated controls (Group 1) and lower in Groups 2 3 and 4 in which growth was induced Activity of this enzyme was also high in muscle of normal animals of Group 5 in which gain in weight was small due to paired feeding against hypophysectomized controls it was low in Group 6 consisting of normal animals fed *ad libitum* and growing rapidly

GLUTAMIC OXALACETIC TRANSAMINASE
DATA OF BARTLETT AND GLYNN

GROUPS OF RATS	GROWTH HORMONE γ/10 DA	GAIN IN WEIGHT	LIVER WT GRAMS	GL-OX-TR $Q^{10}T$ MUSCLE	LIVER
1 HC	0	1 2	2 83	297	29
2 HT	275	14 4	2 38	247	33
3 HT	600	16 0	2 45	249	42
4 HT	1000	16 4	2 50	242	57
5 NC	0	5 9	2 47	275	42
6 NC	0	74 8	6 79	229	30

GROUPS 1 AND 6 FED AD LIBITUM
GROUPS 2 3 4 AND 5 PAIRED FED AGAINST GROUP 1

FIG 2

GROWTH HORMONE AND LIVER ENZYMES
DATA OF BEATON OZAWA BEATON
AND McHENRY

GROUPS	ADULT MALE RATS		YOUNG MALE RATS	
TREATMENT	SALINE	GROWTH HORMONE	SALINE	GROWTH HORMONE
Q_{UREA}	4 99	3 77	2 99	2 83
ARGINASE	83	74	71	71
TRANSAMINASES -				
GLUTAMIC - OXALACETIC	157	157	138	131
GLUTAMIC - PYRUVIC	120	101	60	90

Fig 3

In liver as well as in muscle the general picture was one of an inverse relationship between glutamic oxalacetic transaminase activity and change in weight In the hypophysectomized treated rats (Groups 2 3 and 4) liver weight decreased and activity of the enzyme was high in normal animals fed *ad libitum* (Group 6) liver weight like body weight increased rapidly and activity of the enzyme was low Cohen and Hekhuis[15] called attention to the low transaminase activity of rapidly growing embryonic malignant or regenerating tissues Changes observed during induced growth would seem to extend this relationship An important experimental condition in the experiments of Bartlett and Glynn is that the animals were sacrificed after a 10-day period of treatment Since nitrogen storage occurs very promptly it could have preceded the change in enzyme activity

In this connection experiments of the Toronto group[16] presented in Figure 3 are of interest Large single injections of growth hormone—3 mg per 100 grams body weight—were administered to rats which were sacrificed only 3 hours later Each of the two groups—adult males and young males—was made up of control animals injected with saline and experimental ones receiving growth hormone In the adult male group the rate of urea formation by liver slices and the activities of hepatic arginase and glutamic pyruvic transaminase were reduced by growth hormone All of these changes would seem to favor diminished catabolism of amino acids They were not observed in young male rats in which growth hormone has little effect In fact the activity of glutamic pyruvic transaminase increased

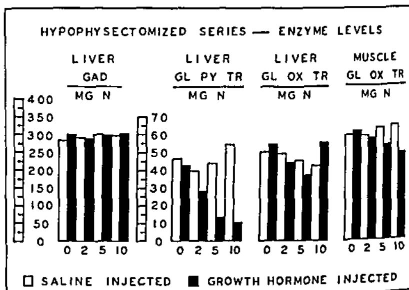


FIG 4

in this group Glutamic oxalacetic transaminase in liver was unaffected in either group

Mathies and I studied the activity of glutamic acid dehydrogenase during induced growth [17] In hypophysectomized rats fed *ad libitum* the activity of this enzyme per gram of liver fell during rapid growth induced by large doses of growth hormone although the amount in the entire organ increased slightly Synthesis of the enzyme apparently did not keep up with growth of the rest of the organ The decrease in activity per gram of liver was not observed after small doses of growth hormone which did cause gain in weight It was not regarded therefore as an essential prerequisite for growth

Zuchlewski[18] has recently carried out a detailed study of this entire group of enzymes under uniform experimental conditions L glutamic acid dehydrogenase and glutamic pyruvic transaminase in liver as well as glutamic oxalacetic transaminase in liver and muscle were determined in 40 hypophysectomized and 40 sham operated rats Each series of 40 was subdivided into two groups of 20 receiving injections of saline and growth hormone respectively The groups of 20 were further subdivided into 4 subgroups, one sacrificed at the start and three after treatment for 2 5 and 10 days respectively In Figures 4 to 6 black bars represent average values in 4 to 7 animals receiving 100 gamma of growth hormone per day and unshaded bars the averages in a similar number of rats injected with saline Transaminase values are expressed in micromoles of pyruvate or oxalacetate formed per hour per milligram of nitrogen calculated from a 10 minute period Glutamic acid dehydrogenase figures represent micromoles of oxygen uptake per hour per milligram of nitrogen calculated from alpha

ketoglutarate formed in 20 minutes Both the hypophysectomized series of rats and the sham-operated ones (designated as normal in the figures) were fed *ad libitum*

Results in the hypophysectomized series appear in Figure 4 Glutamic acid dehydrogenase in liver was unaffected by treatment with growth hormone confirming our earlier findings Glutamic pyruvic transaminase activity in liver decreased in a striking and progressive manner This result indicates how rapid and extensive changes in activity may be Glutamic oxalacetic transaminase in liver decreased at first then increased again In muscle the fall in activity of this enzyme observed by Bartlett and Glynn was confirmed Since glutamic pyruvic and glutamic oxalacetic transaminases require the same coenzyme it appears likely that the changes in activity which differ widely for the two enzymes represent differences in apoenzyme concentrations

The significance of results in the hypophysectomized series can best be evaluated if they are seen side by side with the corresponding findings in the normal series in which growth hormone produced no significant changes in activity of any of these enzymes With respect to glutamic oxalacetic transaminase in liver (Fig 5) one can only conclude that results are more variable in the hypophysectomized rats than in the normal ones In muscle the findings are consistent and confirm Bartlett s view that the characteristic change during induced growth is a reduction of the abnormally high activity in hypophysectomized animals to the normal one of growing animals

Zuchlewski s most striking finding—reduction of glutamic pyruvic

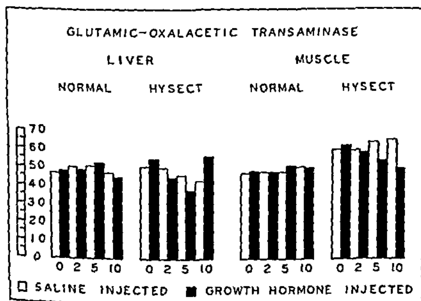


Fig 5

transaminase in the liver—is presented in Figure 6, together with data on gains in weight In the normal group no change in activity of this enzyme occurred although the animals gained 30 grams in 10 days as against 20 grams in the case of the hypophysectomized treated group The decrease in activity of glutamic pyruvic transaminase cannot be therefore, an essential prerequisite for growth under all circumstances It may be a feature of growth under these particular conditions of nutrition and replacement therapy

Average weights and food consumption of the hypophysectomized and normal rats which received growth hormone are tabulated in Figure 7 In the hypophysectomized rats food consumption per 100 grams of body weight increased with the duration of treatment, reaching 6 53 grams per day at the end of the 10 day period In normal animals of the same age it decreased with increasing weight but even so was 7 28 grams at the corresponding time While a deficit of 10 per cent in food intake is significant it alone can hardly account for the drastic decrease in glutamic pyruvic transaminase activity Throughout our studies decreases in enzyme activity have been related to the changes in growth rate rather than to food intake For example the relative decrease in glutamic acid dehydrogenase activity [17] produced by large doses of growth hormone occurred in rats fed *ad libitum* in which the resulting increase in growth rate was maximal The decrease in glutamic pyruvic transaminase activity of liver likewise occurred in treated hypophysectomized rats fed *ad libitum* in which the

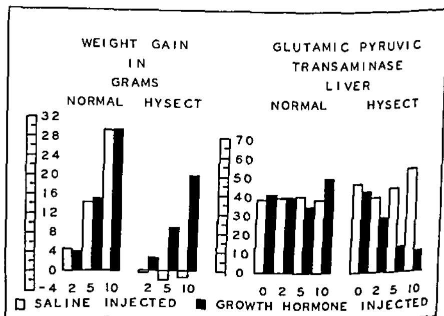


Fig 6

DAYS TREATED	NO OF RATS	AVERAGE FINAL WEIGHT GM	AVERAGE DAILY FOOD GM	FOOD INTAKE PER 100 GM
HYPOPHYSECTOMIZED SERIES				
2	5	65 6	3 86	5 88
5	6	72 0	4 66	6 47
10	5	85 0	5 55	6 53
NORMAL GROWING SERIES				
2	5	96 0	8 25	8 58
5	6	101 1	7 80	7 72
10	7	117 6	8 57	7 28
ALL ANIMALS RECEIVED 100 γ GROWTH HORMONE PER RAT PER DAY ARMOUR NO K40105R				

Fig 7

change in growth rate was large it was not observed after growth hormone injections in normal animals whose growth rate was not affected by growth hormone (Fig 6)

If one explains the absence of any effect of growth hormone on the growth rate of normal young rats by assuming that its action is already maximal it follows that such maximal physiological action does not diminish glutamic pyruvic transaminase activity in the liver of intact animals The decrease in hypophysectomized rats treated with growth hormone may be due to the fact that replacement therapy is decidedly unbalanced During the sudden spurt in growth depletion of glutamic pyruvic transaminase may occur as a result of the rapid synthesis of other proteins Selective depletion of enzymes is known to occur during protein restrictions in fact Meikelham et al[19] have attempted to correlate the differences in lability of enzymes with their cytochemical distribution

Whatever the explanation of the decrease in glutamic pyruvic trans aminase may be one cannot overlook the fact that it occurred and that it might influence the metabolism of alanine, pyruvic acid and other com pounds Preliminary experiments on the metabolism of alanine labeled with N^{15} in hypophysectomized rats are presented in Figure 8 The treated group received 100 gamma of growth hormone daily for 5 days controls received an equal volume of saline solution On the third fourth and fifth days both groups were given intraperitoneal injections of N^{15} labeled alanine There were two findings of interest When hydrolysates of blood fibrin or hydroly sates of proteins from liver spleen kidney or heart were compared the animals treated with growth hormone always showed slightly greater incor poration of N^{15} than was noted in untreated animals Secondly when liver

INCORPORATION OF N^{15} FROM LABELED ALANINE

FRACTION		CONTROL GROUP AT % XS	TREATED GROUP AT % XS
LIVER –			
PROTEIN HYDROLYSATE		031	037
BUTANOL EXTRACT		031	036
TYROSINE		020	022
WATER PHASE		035	040
GLUTAMIC ACID			079
BLOOD FIBRIN HYDROLYSATE		044	052
SPLEEN PROTEIN	"	039	042
KIDNEY	"	034	041
HEART	"	024	033

FIG 8

protein hydrolysate was fractionated by the Dakin method atom per cent excess of N^{15} was similar in the butanol extract and the water phase The former contains mono amino mono carboxyllic acids and proline the latter the dicarboxyllic and basic amino acids Tyrosine which separated was labeled and glutamic acid isolated from the water phase obtained from liver protein of the treated group was heavily labeled These studies are preliminary but they suggest that the distribution of N^{15} from labeled alanine was probably not greatly altered

Discussion has been limited to changes in tissue enzyme activity which are statistically significant as indicated by data in the cited publications and thesis It may be added that nitrogen content per gram of tissue was virtually constant in the experiments presented in Figures 4 5, and 6 so that the conclusions would have been the same whether the results had been expressed per mg of nitrogen or per gram of fresh tissue

Summary

To summarize the changes in activity of alkaline phosphatase in tibia L glutamic acid dehydrogenase and glutamic pyruvic transaminase in liver and glutamic oxalacetic transaminase in liver and muscle have been studied in some detail with concordant results We have no evidence that any of these enzymes should be regarded as chemical mediators through which the pituitary exerts its control of growth Some of the changes observed may be important however in the growth process Activity of alkaline phosphatase of tibia decreases after hypophysectomy while that of glutamic

oxalacetic transaminase of muscle increases and treatment with growth hormone reverses the change in either case Similar changes in activity of these enzymes are seen in the intact animal during development or during interruption and resumption of growth Less impressive is the relative decrease in L glutamic acid dehydrogenase of liver produced only by large doses of growth hormone It did not occur in hypophysectomized animals fed *ad libitum* and treated with moderate doses of hormone or in rapidly growing normal animals

More striking than any of the other changes is the reduction of glutamic pyruvic transaminase in liver of hypophysectomized rats treated with moderate doses of growth hormone and fed *ad libitum* The possibility is considered that with food intake somewhat below normal and replacement therapy decidedly unbalanced this enzyme may be depleted as a result of rapid synthesis of other protein Preliminary experiments indicate that diminished activity of this enzyme in liver did not produce any radical change in transfer of amino groups from alanine to other amino acids

References

1 Fraenkel Conrat H Simpson M E and H M Evans *Am J Physiol* **138** 439 (1943)
2 Levin L *Am J Physiol* **141** 143 (1944)
3 Gaebler O H *Symposium on Protein Metabolism* Nutrition Symposium Series No 8 New York The National Vitamin Foundation Inc March 38 (1954)
4 Kay H D *Biochem J* **20** 791 (1926)
5 Robison R and A H Rosenheim *Biochem J* **28** 684 (1934)
6 Buchwald K W and L Hudson *Endocrinology* **41** 111 (1947)
7 Mathies J C and O H Gaebler *Endocrinology* **45** 129 (1949)
8 Marx W Simpson M E and H M Evans *Endocrinology* **30** 1 (1942)
9 Mathies J C Goodman E D and L Palm *Am J Physiol* **168** 352 (1952)
10 Li C H Kalman C Evans H M and M E Simpson *J Biol Chem* **163** 715 (1946)
11 Becks H Simpson M E Evans H M Ray R D Li C H and C W Asling *Anat Rec* **94** 631 (1946)
12 Kochakian C D *Annals of the New York Academy of Sciences* **54** 534 (1951)
13 Braunstein A E and R M Asarkh *J Biol Chem* **157** 421 (1945)
Braunstein A E and S M Bychkov *Nature* **144** 751 (1939)
14 Bartlett P D and M Glynn *J Biol Chem* **187** 253 (1950) *J Biol Chem* **187** 261 (1950)
15 Cohen P P and G L Hekhuis *Cancer Research* **1** 620 (1941)
16 Beaton G H Ozawa G Beaton J R and E W McHenry *Proc Soc Exp Biol Med* **83** 781 (1953)
17 Gaebler O H and J C Mathies *Endocrinology* **51** 469 (1952)
18 Zuchlewski A Ph D Thesis Wayne University Graduate School 1954
19 Meikleham V Wells J C Richert D A and W W Westerfeld *J Biol Chem* **192** 651 (1951)
20 Schoenheimer R and S Ratner *J Biol Chem* **127** 301 (1939)

23

The Effect of Insulin and Alloxan Diabetes on the Transport of Glucose and Other Sugars into the Cells of Muscle and Brain

*C R Park**
Vanderbilt University School of Medicine Nashville Tennessee

It is well established that insulin accelerates glucose uptake and utilization by muscle (see reviews by Krahl[1] and Stadie) There is also evidence that hormonal factors resulting from adrenal cortical and anterior pituitary activity in alloxan diabetes inhibit glucose uptake by this tissue[3 4 5] While the nature of these diabetogenic substances is not entirely clear it appears likely that the growth hormone is one of the factors involved[6 7 8] In brain tissue insulin and these diabetogenic factors appear to have no effect (see Himwich[9])

The present paper is concerned with the problem of determining what step in the metabolism of glucose by muscle is affected by these hormonal factors The findings in muscle are compared to those in the brain The data presented here are from our own largely unpublished studies † More complete presentation of the background of this problem will be found in the recent review by Stadie[2]

Levine Goldstein Huddlestun Henry and Klein[10 11] have recently proposed that transport across the cell membrane may be the rate limiting step for glucose utilization by certain tissues They propose that insulin accelerates transport thus making glucose available in larger amounts for subsequent metabolism These proposals were based on the finding that certain non metabolizable sugars in the absence of insulin were confined to the extracellular water in eviscerated nephrectomized dogs With insulin

* The data presented in this report have been collected in collaboration with L H Johnson J Wright and H Batsel

† Preliminary reports of part of this work have been presented elsewhere [2] [5]

administration however these sugars entered the intracellular water These observations with non metabolizable sugars have been confirmed and extended by Wick and Drury [13,14] Ross [1] Haft and associates[16] and Fisher and Lindsay [17] using a variety of test systems Levine s proposal that glucose transport would be accelerated by insulin was based in part on the observation that those sugars sensitive to insulin had the same configuration as glucose in carbon atoms 1 2, and 3 More direct evidence in respect to glucose itself however was not obtained since it could not be determined whether insulin affected transport into the cell or some subsequent step involving glucose utilization

In the present studies it has been possible to distinguish whether insulin affects the transport of glucose or a subsequent metabolic step This distinction was based on the change in the level of intracellular free glucose as will be discussed presently In addition the effect of insulin on the transport of several other sugars has been examined in muscle and brain tissue specifically Our experimental procedure in brief was as follows Sprague Dawley rats fasted 18 hours were eviscerated just before use in order to avoid insulin secretion The sugar to be tested was infused intravenously at a steady rate for a period of two hours with or without added insulin At the end of this time the concentration of sugar was determined simultaneously in the blood serum muscle tissues and brain The ratio of the concentration in the tissue to the concentration in the blood serum was regarded as an index of whether or not the sugar had remained in the extracellular fluid or had entered the cells This interpretation may be understood more easily with reference to Figure 1

In this figure the ratio of the tissue to serum free sugar concentrations ($[S]_T/[S]_S$) is shown by the height of the columns It would be expected that low ratios would be found if the sugar were confined to the extracellular water since the extracellular water is only a small fraction of the total tissue water [18,19,0] The horizontal broken lines across each column indicate the limiting ratio for extracellular distribution which would be obtained when the sugar concentration in the extracellular water reached the blood serum level This value is higher for the heart because of its larger extracellular volume [19,21] On the other hand if the sugar could pass across the cell membrane into the relatively large volume of intracellular water ratios above the extracellular limiting value would be expected * If the concentration

---

In theory a ratio higher than the intracellular limiting value could also be obtained if the sugar became more concentrated in the interstitial water than in the serum or if the extracellular volume were expanded The first possibility is most unlikely in view of the generally accepted concept that sugars pass in and out of the capillaries by a process of diffusion In regard to the second possibility the large magnitude of the effect seen with insulin in the present studies would require a 2 to 4 fold expansion of the extracellular volume in muscle in the absence of any large exogenous supply of water and salt Such extensive shifts in water and electrolytes within the body if they are indeed possible have never been associated with the action of insulin

throughout the tissue for example, rose to equal the serum concentration a ratio of 1 0 would be found

The first group of experiments is concerned with the effect of insulin on the transport of four non metabolizable sugars In the panel on the left in Figure 1 are shown the ratios obtained when galactose or galactose plus insulin was infused into eviscerated rats Galactose concentrations were determined by a quantitative paper chromatography in order to ensure specificity In the absence of insulin the concentrations of galactose found in the diaphragm heart and gastrocnemius were very low relative to the serum With insulin administration the ratios were much higher than would be possible with an extracellular distribution Since galactose does not arise by metabolic reactions within the cell insulin must therefore accelerate its

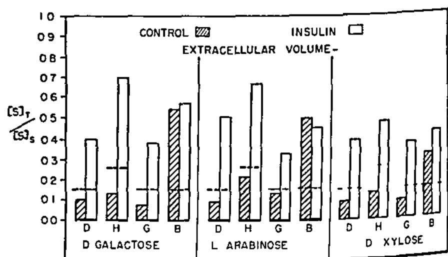


FIG 1 The effect of insulin on the distribution of D galactose L arabinose and D xylose in the tissue water of muscle and brain in eviscerated rats

Abbreviations $(S)_T/(S)_S$ the ratio of the concentrations of the free sugar in the tissue to the concentration of the sugar in the blood serum (see text for interpretation) D diaphragm H heart G gastrocnemius B brain

Procedure Fasting rats were eviscerated and then given a continuous infusion of the sugar to be tested with or without added insulin for a period of 2 hours The sugar was dissolved in 0 9 per cent NaCl and injected in a volume of 1 ml per 100 g of rat Fifteen minutes after the infusion was stopped the rat was killed and samples of blood serum muscle and brain were taken as rapidly as possible for estimation of their sugar content Galactose was estimated by quantitative paper chromatography using a modification of the methods of Partridge[7] L Arabinose and D xylose were estimated by a modification[8] of the orcinol method of Mejbaum[9] The average concentration of the free sugar in the water of the blood serum and tissues was then calculated using the values 94 per cent and 76 per cent for the water content of the serum and tissues respectively[18]

In each pair of values for the muscles the ratio with insulin is significantly different from the control (p<0 1%)

transport into the cell from the outside In the brain however the ratios exceeded the limits of an extracellular distribution in the presence or absence of insulin It appears therefore that galactose enters these cells in the absence of insulin and that the rate of entrance is not accelerated by the hormone

Similar ratios were obtained with the pentose L arabinose as shown in the center panel of Figure 1 The distribution of this sugar was apparently extracellular in the absence of insulin but largely intracellular in the presence of insulin Penetration into the cells of the brain occurred at a significant rate without insulin and was not accelerated by the hormone The results obtained with the pentose D-xylose were essentially the same (Fig. 1 right panel)

In contrast to the above findings insulin had little or no effect on the transport of D-ribose into the tissue cells (Fig 2 left panel) The tissue to serum ratios were low in the absence of insulin and were not increased significantly or consistently by the hormone It was important to be sure that the apparent lack of ribose penetration was not due in fact to removal of

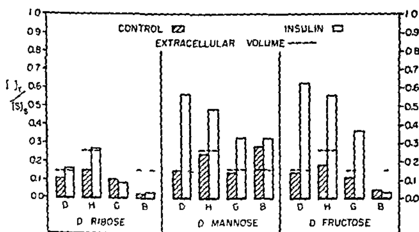


Fig 2 The effect of insulin on the distribution of D-ribose D mannose and D-fructose in the tissue water of muscle and brain of eviscerated rats

Abbreviations $(S)_T/(S)_S$ the ratio of the concentrations of the free sugar in the tissue to the concentration of the sugar in the blood serum (see text for interpretation) D diaphragm H heart G gastrocnemius B brain

Procedure Fasting eviscerated rats were infused intravenously with the sugar to be tested as described in Figure 1 D Ribose was estimated by a modification[28] of Mejbaum s procedure [9] D-mannose was estimated by quantitative paper chromatography adapted from Partridge [7] D-fructose by the procedure of Roe[35] or Hendrick [38]

In the case of D-ribose the values with insulin are not significantly different from the appropriate controls In the case of D mannose and D-fructose the values with insulin are significantly different ($p<0.1\%$) in the case of the muscle tissues but not the brain

the sugar by intracellular metabolic processes Total recoveries of the pentose from the eviscerated animal therefore were carried out and virtually all the sugar injected could be recovered at the end of the experiment The present results do not mean necessarily that ribose transport is completely unresponsive to insulin It may simply be that the rate does not become fast enough to be detected at the time interval tested Ribose and a number of other sugars [3 4] which penetrate cells very slowly do not differ significantly in size and solubility from sugars which enter very much faster Since size and lipid solubility are the principal known factors governing diffusion into cells [4] the relatively slow penetration of ribose suggests that transport across the cell membrane may not be a process of simple diffusion It points rather to an enzymatic or chemical process sensitive to changes in configuration as suggested by Levine and associates [11 1] Wick and Drury[13 14] and Ross [5 6]

In the second group of experiments it was shown that insulin accelerates the transport of the metabolizable sugars D mannose and D fructose into muscle cells The experimental procedure was the same as already described The ratios shown in Figure 2 (center panel) indicate that mannose is extracellular in muscle in the absence of insulin but that large amounts of the free sugar are intracellular in the presence of insulin Mannose is found within the brain cells to about the same extent in the presence or absence of the hormone

In their studies with the eviscerated dog Levine and associates[11 12] postulated that mannose was converted to glucose prior to entry into the cells In the present studies however there can be no doubt that mannose penetrates the cells without conversion since the sugar recovered in the tissue extracts was identified as mannose by quantitative paper chromatography Drury and Wick[30] have also concluded that mannose as such penetrates the extrahepatic cells of the eviscerated rabbit

There has been some question as to whether insulin affects fructose in muscle tissue [16 30 31 3 33] The present data (Fig 2) indicate clearly that fructose is extracellular in the absence of insulin but is present as the free sugar in large amounts in the presence of insulin In the brain very small amounts of fructose were recovered It cannot be determined by the present procedure to what extent this was due to slow penetration[33] or to removal of the sugar by brain hexokinase [34] Cori and associates[31] found that insulin did not increase the utilization of fructose by the eviscerated rat In the present experiments when fructose was estimated in the whole carcass at the end of the experiment about 20 per cent more of the sugar was utilized in the insulinized animals By comparison when glucose was administered insulin promoted utilization by 400–500 per cent While insulin makes fructose available in larger amounts as substrate within the cell phosphorylation is not greatly increased This may be due to the very low affinity of muscle fructokinase for its substrate [34]

The third group of experiments deals in some detail with the effect of insulin on the transport of glucose, the sugar of principal physiological interest The procedure was as already described except that the period of infusion of glucose was only one hour In Figure 3 is shown the effect of insulin on the glucose concentration in the heart muscle of eviscerated rats At any given serum glucose level the free glucose concentration found in the muscle was relatively low in the absence of insulin In the presence of insulin at comparable blood glucose levels the concentration in the muscle was 3 to 4 times higher The determinations for the control and hormone treated animals fit fairly well along two straight lines showing that the ratios of the muscle to serum glucose concentrations were reasonably constant and independent of the absolute level of the blood glucose

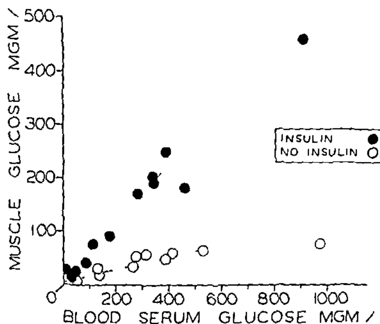


Fig 3 The effect of insulin on the concentration of free glucose in the heart muscle of eviscerated rats at various blood serum glucose levels

Fasting rats were eviscerated and then given a continuous intravenous infusion of glucose with or without insulin (10 units per 100 g) for a period of 1 hour Fifteen minutes later the rat was killed and samples of blood serum and heart muscle were taken as rapidly as possible for estimation of free glucose content Various levels of blood serum glucose were obtained by varying the concentration of glucose infused

The methods for extraction of glucose and removal of glucose phosphates[39] did not cause breakdown of glucose phosphates as shown by appropriate controls Free glucose in the extracts was determined by the reduction of triphosphopyridine nucleotide in a system containing yeast hexokinase and Zwischenferment The free glucose concentrations were corrected for the protein content of the tissue as described under Figure 1

These data for the heart and similar data for the diaphragm and gastrocnemius were used to calculate the muscle to serum free glucose concentrations ($[G]_M/[G]_S$) shown on the left in Figure 4 In the absence of insulin the ratios indicated an extracellular distribution In the presence of insulin however there was a large amount of intracellular free glucose in the diaphragm and heart The effect of insulin in the gastrocnemius was relatively small put probably significant

Since evisceration causes extensive circulatory and metabolic changes it appeared important to determine whether the same effect of insulin could also be obtained in intact rats (Fig 4 center) In the control animals the ratios were determined only at normal blood glucose concentrations since

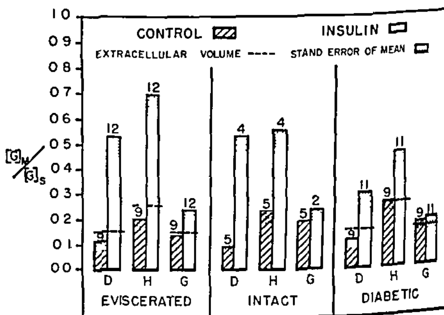


FIG 4 The effect of insulin on the distribution of free glucose in the muscles of eviscerated intact and alloxan diabetic rats

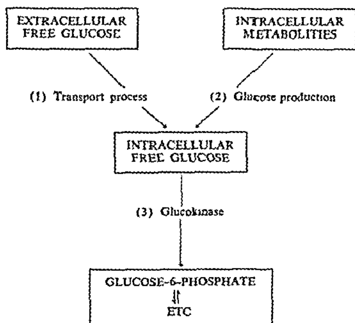
Abbreviations $(G)_M/(G)_S$ the ratio of the concentration of free glucose in the muscle to the concentration in the blood serum (see text for interpretation) D diaphragm H heart G gastrocnemius The number of experiments in each series is given by the figure at the top of each column

The experimental procedure and analytical methods have been outlined under Figure 3 The control rats in the intact and diabetic series were infused with saline only All diabetic rats had 18 hour fasting blood sugars of 250 mg per cent or more 2–4 days after intravenous injections of 60 mg of alloxan per kg body weight

The statistical analysis of the insulin effect for significance in terms of p values was as follows (per cent) In the eviscerated series diaphragm $<0\ 1$ heart $<0\ 1$ gastrocnemius <1 in the intact series diaphragm $<0\ 1$ heart $<0\ 1$ gastrocnemius >1 in the diabetic series diaphragm $<0\ 1$ heart $<0\ 1$ gastrocnemius >1

higher concentrations might cause insulin secretion The ratios in the absence of insulin indicated an extracellular glucose distribution With insulin administration however free glucose accumulated within the cells of the diaphragm and heart as was seen in the previous series

The rise in intracellular free glucose in the presence of insulin has been interpreted as follows The level of intracellular glucose has been considered to be a function of three processes as shown in the following diagram



The level of intracellular glucose would rise as a result of one or more of the following changes in the rate of these processes (1) acceleration of transport into the cell (2) reduced phosphorylation by the glucokinase reaction which is the first step in glucose metabolism In regard to the action of insulin the last two changes appear most unlikely on the following grounds Glucose production by muscle is generally regarded as being slight due to the absence of glucose phosphatase and because the gluco kinase equilibrium strongly favors glucose 6 phosphate formation The only reactions known to release free glucose in muscle are associated with glycogen breakdown[37 38] With insulin however there is a strong stimula tion of glycogen synthesis Furthermore in experiments with the isolated rat diaphragm[39] we could demonstrate no effect of insulin on glucose production The muscle was incubated in medium containing C^{14} labelled glucose and after an appropriate time the free glucose of the muscle was isolated In no instance did insulin cause a dilution of the isotope indicating that no free glucose was liberated from endogenous unlabelled sources The

possibility that phosphorylation is reduced is contrary to the well-established fact that insulin increases glucose utilization which must necessarily pass through the glucokinase step On these grounds therefore it seems that the only reasonable explanation for the rise in intracellular free glucose is the acceleration of transport into the cell This interpretation is consistent with the observations in regard to galactose, L arabinose, xylose mannose and fructose Insulin must accelerate the transport of these sugars since the hormone does not increase their intracellular production or inhibit their utilization

The present studies indicate that the action of insulin on transport is distinct from any possible effect on the glucokinase system as postulated by Cori and associates[40 41 4 43] First insulin accelerates transport of such sugars as galactose L arabinose and xylose which are not phosphorylated by the enzyme Second in the case of glucose the intracellular free sugar would fall rather than rise if the acceleration of transport were secondary to stimulation of glucokinase activity It also follows on the same grounds that glucokinase is not an integral part of the transport system It may be further concluded that transport is the rate limiting step for glucose utilization or uptake by the non insulinized muscle This follows from the observation that no intracellular glucose is found in the absence of the hormone The enzyme systems subsequent to transport are limited therefore by the rate at which transport makes substrate available With acceleration of transport more substrate becomes available and the activity of glucokinase and subsequent enzyme systems in the metabolic sequence is increased With full insulinization and abundant free intracellular glucose the glucokinase reaction may become the rate limiting step

The final group of experiments in which glucose with or without insulin was infused into alloxan diabetic rats is presented in Figure 4 (right panel) In the absence of insulin the tissue to serum glucose ratios indicated little or no free intracellular glucose With insulin administration however intracellular free glucose accumulated in the diaphragm and heart No effect was seen in the gastrocnemius As mentioned earlier glucose utilization is inhibited in alloxan diabetes by diabetogenic factors which result from anterior pituitary and adrenal cortical activity The absence of free intracellular glucose in the diabetic muscle indicates that here also the transport process rather than the glucokinase reaction[44] is the rate limiting step for glucose utilization Since utilization is inhibited it follows that the transport step must be the site of inhibition by the diabetogenic factors If the inhibition were on the hexokinase step as was suggested by Cori and associates glucose would still be expected to enter the cell where it would accumulate due to reduced phosphorylation The accumulation of intracellular glucose which occurs when insulin is given indicates that the inhibition of transport can be counteracted by the hormone Insulin however is somewhat less effective than in the normal animal

In discussing the action of insulin on the transport of glucose the possibility that insulin affects the capillary circulation or permeability must be considered A more rapid flow of glucose out of the capillaries could conceivably result in higher concentrations of glucose at the cell wall and hence faster transport into the cell There is no evidence however that insulin affects capillary circulation While capillary permeability may be affected by insulin, it would be difficult to believe that this is a major action of the hormone The studies of Levine and associates[10, 11, 1] and Wick and Drury[15, 16] show that sugars including glucose are distributed relatively rapidly throughout the extracellular water in the absence of the hormone There would appear therefore to be no major barrier to glucose penetration until the cell wall is reached In addition insulin effects on glucose utilization are readily observed in *in vitro* systems such as the lens of the eye [1] where capillary effects can play no role There seems to be little doubt therefore that the principal locus of this insulin action is at the cell membrane

In the present studies with glucose it was observed that the effect of insulin on free glucose in the gastrocnemius was small or absent although large in the heart and diaphragm This difference is most easily explained on the basis of a quantitatively smaller effect on transport rather than a qualitatively different action of the hormone In the earlier discussion it was pointed out that any acceleration of transport should result in a rise in intracellular free glucose However a large or readily measurable rise would be expected only when the transport rate increased to such an extent that the capacity of the glucokinase system to remove intracellular glucose was exceeded In other words a large rise would occur only when the glucokinase system approached saturation with respect to substrate This situation did not occur in the gastrocnemius perhaps because the intrinsically smaller capillary bed and restricted diffusion due to immobility of the muscle made less glucose available for transport through the cell wall It is also possible that the capacity of the glucokinase system in the gastrocnemius is greater than in the heart or diaphragm This would allow faster phosphorylation of intracellular free glucose and reduce its accumulation In the case of those sugars which are removed slowly if at all by intracellular metabolism the effect of insulin in the gastrocnemius was apparently the same as in the diaphragm and heart although somewhat smaller

The passage of glucose into the muscle cell does not appear to be a process of simple diffusion As mentioned earlier the widely different rates of transport of sugars which are very similar in size and solubility suggests a more specific process The effect of insulin on some sugars and not on others such as sorbitol[16] and possibly ribose would also be difficult to reconcile with a process of diffusion The exact relationship between structure transport rate and insulin effect is not yet clear It may be noted here that the present studies with mannose and fructose do not support Levine and asso

ciates conclusion that a glucose configuration in carbon atoms 1, 2 and 3 is necessary for an insulin effect on transport A further argument against a diffusion process is the observation that the transport process in the brain apparently differs from the muscle in being insensitive to insulin The failure of insulin to stimulate transport into the brain is consistent with the well known absence of any insulin effect on glucose metabolism by this tissue

In their recent studies of the human erythrocyte LeFevre[47 48 49] and Widdas[50 1] have obtained some indications of the nature of the process by which glucose and other sugars cross the cell membrane They point out from kinetic studies that transport cannot be a process of simple diffusion Furthermore the transport rate is very sensitive to changes in the configuration of the sugar molecule, and competition for transport among sugars including glucose fructose and galactose can be demonstrated It was particularly interesting that the kinetics of transport fit well to the postulate that glucose crosses the membrane in combined form This concept of carrier transport has many analogies to an enzyme catalyzed process

In summary the present studies provide experimental support for the proposal that an action of insulin in muscle is to accelerate the transport of glucose across the cell membrane Acceleration of transport makes glucose available in larger amounts within the cell for subsequent metabolic steps The transport of a number of other sugars including mannose and fructose is similarly accelerated Transport into the cell is the rate limiting step for glucose utilization by the non insulinized muscle, and it is inhibited in alloxan diabetes Insulin does not appear to affect the transport of sugars into the cells of the brain

References

1 Krahl M E *Ann N Y Acad Sci* **54** 649 (1951)
2 Stadie W C *Physiol Rev* **34** 52 (1954)
3 Krahl M E and C F Cori *J Biol Chem* **170** 607 (1947)
4 Villee C A and A B Hastings *J Biol Chem* **179** 673 (1949)
5 Krahl M E and C R Park *J Biol Chem* **174** 939 (1948)
6 Park C R Brown D H Cornblath M Daughaday W H and M E Krahl *J Biol Chem* **197** 151 (1952)
7 Park C R *Phosphorus Metabolism* Vol II Eds McElroy W D and B Glass Baltimore Johns Hopkins Press 1952 634
8 Bornstein J and C R Park *J Biol Chem* **205** 503 (1953)
9 Himwich H E *Brain Metabolism and Cerebral Disorders* Baltimore Williams and Wilkins 1951
10 Levine R Goldstein M S Huddlestun B and S P Klein *Am J Physiol* **163** 70 (1950)
11 Goldstein M S Henry L Huddlestun B and R Levine *Am J Physiol* **173** 207 (1953)
12 Levine R and M S Goldstein *Brookhaven Symposia in Biology* **5** 73 (1952)
13 Wick A N and D R Drury *Am J Physiol* **166** 421 (1951)

14 Wick A N and D R Drury *Am J Physiol* **173** 229 (1953)
15 Ross E J *Nature* **171** 125 (1953)
16 Haft D I Mirsky A and G Persutti *Proc Soc Exp Biol Med* **82** 60 (1953)
17 Fisher R B and D B Lindsay *J Physiol* **124** 20P (1954)
18 Newman E V *Am J Physiol* **122** 359 (1938)
19 Manery J F and A B Hastings *J Biol Chem* **127** 657 (1939)
20 Nichols G Jr Nichols N Weil W B and W M Wallace *J Clin Invest* **32** 1299 (1953)
21 Nichols G Jr Personal communication
22 Kozawa S *Biochem Ztschr* **60** 231 (1914)
23 Wilbrandt W *Ergebn Physiol* **40** 204 (1938)
24 Danielli J F in Davson H and J F Danielli *Permeability of Natural Membranes* Chap VIII Cambridge Univ Press 1952
25 Ross E J *J Physiol* **112** 229 (1951)
26 Ross E J *J Physiol* **116** 414 (1952)
27 Partridge S M *Nature* **164** 443 (1949)
28 Horecker B L and P Z Smyrniotis *J Biol Chem* **193** 371 (1951)
29 Mejbaum W *Z physiol Chem* **258** 117 (1939)
30 Drury D R and A N Wick *Am J Physiol* **177** 535 (1954)
31 Cori C F and G T Cori *Proc Soc Exp Biol Med* **26** 432 (1929)
32 Gammeltoft A Kruhoffer P and E Lundsgaard *Acta Physiol Scand* **7** 209 (1944)
33 Mackler B and G M Guest *Proc Soc Exp Biol Med* **83** 327 (1953)
33a Geiger A Magnes J Taylor R M and M Veralli *Am J Physiol* **177** 138 (1954)
34 Slein M W Cori G T and C F Cori *J Biol Chem* **186** 763 (1950)
35 Roe J H *J Biol Chem* **107** 15 (1934)
36 Kendrick A B *Federation Proc* **11** 239 (1952)
37 Cori G T Closs J O and C F Cori *J Biol Chem* **103** 13 (1933)
38 Cori G T and J Larner *J Biol Chem* **188** 17 (1951)
39 Park C R Bornstein J and R L Post To be published
40 Price W H Slein M W Colowick S P and G T Cori *Federation Proc* **5** 150 (1946)
41 Cori C F *Harvey Lectures Ser* **41** 253 (1945–1946)
42 Colowick S P Cori G T and M W Slein *J Biol Chem* **168** 583 (1947)
43 Cori C F *First International Congress Biochem* 9 (1949)
44 Krahl M E and C F Cori *J Biol Chem* **170** 607 (1947)
45 Wick A N Drury D R and E M McKay *Am J Physiol* **163** 224 (1950)
46 Drury D R and A N Wick *Am J Physiol* **166** 159 (1951)
46a Wick A N Drury D R and E M McKay *Ann N Y Acad Sci* **54** 684 (1951)
47 LeFevre P G *J Gen Physiol* **31** 505 (1948)
48 LeFevre P G and M E LeFevre *J Gen Physiol* **35** 891 (1952)
49 LeFevre P G *Federation Proc* **12** 84 (1953)
50 Widdas W F *J Physiol* **118** 23 (1952)
51 Widdas W F *J Physiol* **125** 163 (1954)
52 Park C R *J Clin Invest* **32** 593 (1953)
53 Park C R and L H Johnson *Abstracts of the XIX International Physiological Congress* September 661 (1953)
54 Park C R *Federation Proc* **13** 108 (1954)

DISCUSSION

Growth Hormone and Cellular Systems

Designated Discussion

CARL CORI (Chairman) This discussion period was to be opened by Dr Gerty Cori Regrettably she was unable to come and Dr Lehninger kindly agreed to be the substitute designated discussor He arrived for the symposium but unfortunately became ill and will be unable to fill his role Therefore the problem of a *pinch hitter* arises and I would ask for volunteers

(*Editor s note* By acclamation Dr Carl Cori became the designated discussor)

CARL CORI One of the broad questions which we have been hearing about this morning is whether or not hormones might be regarded as regulators of enzymatic rates If that is their function then we might ask whether or not they influence the specific enzymatic action which would keep them in line with the high specificity of biological processes we are used to at least in the enzyme field We might ask on the other hand whether or not hormones influence a multiplicity of enzymes and therefore act at different points If they have one locus of action then their effects might be considered as being primary secondary or tertiary and their action would require explanation in such a sequence

Now we have heard from Dr Krahl who has introduced another difficulty into the problem of purification of the hormones He mentioned that there may be a lipoprotein in the pituitary which inhibits glucose uptake in the isolated diaphragm and which also inhibits an isolated enzyme, namely hexokinase He reported this lipoprotein factor may be found also, in the serum of diabetic animals If I am not mistaken some attempts have been made by Krahl and also by Bornstein to reverse this inhibition with insulin and perhaps Dr Krahl would like to comment on this point since he didn t mention it in his talk The reversibility by insulin of this inhibition would be an important observation

The second paper that of Dr Gaebler had to do with the maintenance of enzymes in tissues under the influence of injected pituitary hormones The reported studies were based on measurements of enzyme levels and here the important question arises—since so many studies are being made on enzyme concentrations—as to how much of a particular enzyme does the cell need to carry out its function We do not know If there is a series of enzymatic reactions one particular enzyme might be regarded as the rate limiting step and its diminution might effect over all processes However other enzymes may be present in great excess and therefore mask any

possible effect on over all rates This seems to have been the case in one of the transaminases which was diminished under the influence of anterior lobe extract and yet when tagged substrate was administered to test enzymatic activity no marked effects on the metabolic rate were seen So this is the second point we might consider further

The third paper presented by Dr Park had to do with the transport of substances across the cell membrane This phenomenon I think it is fair to say so far has escaped definition or even in any way a mechanistic interpretation The only thing one does say about the transport of glucose and other substances across the cell membrane is that the process is an active one But this doesn t say very much about the obviously important question as to what determines the rate of cellular metabolism Is it determined by the intracellular enzymes themselves or by the substances that cross the cell membrane and their concentration within? Now in the case of oxygen we certainly know that it is not the oxygen tension in the cell which determines the rate of oxidation but rather it is the rate at which the catalysts proceed to use the oxygen According to the data presented by Dr Park however the situation would be reversed It would be the intracellular concentration of glucose which would determine the rate of metabolism This is a very important *point to consider It implies of course that there is a difference* between the extracellular and intracellular glucose which as Dr Park has shown and as recognized for some time is true for muscle In the studies of the liver however measurements of intra and extracellular glucose concentrations have shown them to be equal which follows incidentally from the fact that the liver is not only taking up glucose but also producing it We are faced with the question then as to whether insulin acts at all on the liver Formerly especially by the Soskin Levine school all of the insulin action was placed on the liver and the peripheral action of insulin was not even admitted Now if we accept the proposition which Dr Park has discussed we would reverse our stand and say that insulin acts only on the peripheral tissues not on the liver I would like to point out that in the experiments performed by Levine muscular exercise had exactly the same effect as insulin on the transport of glucose from the extra to the intra cellular space To be remembered also are the experiments by Dr Chaikoff which he recently published in the *American Journal of Physiology* He measured the glucose space in normal and diabetic animals both with and without insulin These animals were entirely normal trained and unanesthetized He could find no effect of insulin on the glucose space and his conclusion was that his data neither denied nor confirmed the concept that insulin acts on glucose transport across the cell membrane I think his statement is fair because you would expect if glucose entry determines the metabolic rate that glucose getting across should be removed just as fast and you would observe no change in its distribution

I would like to direct at Dr Park one comment which seems to be very

DISCUSSION

Growth Hormone and Cellular Systems

Designated Discussion

CARL CORI (Chairman) This discussion period was to be opened by Dr Gerty Cori Regrettably she was unable to come and Dr Lehninger kindly agreed to be the substitute designated discussor He arrived for the symposium but unfortunately became ill and will be unable to fill his role Therefore the problem of a *pinch hitter* arises and I would ask for volunteers

(*Editor s note* By acclamation Dr Carl Cori became the designated discussor)

CARL CORI One of the broad questions which we have been hearing about this morning is whether or not hormones might be regarded as regulators of enzymatic rates If that is their function then we might ask whether or not they influence the specific enzymatic action which would keep them in line with the high specificity of biological processes we are used to, at least in the enzyme field We might ask on the other hand whether or not hormones influence a multiplicity of enzymes and therefore act at different points If they have one locus of action then their effects might be considered as being primary secondary or tertiary and their action would require explanation in such a sequence

Now we have heard from Dr Krahl who has introduced another difficulty into the problem of purification of the hormones He mentioned that there may be a lipoprotein in the pituitary which inhibits glucose uptake in the isolated diaphragm and which also inhibits an isolated enzyme namely hexokinase He reported this lipoprotein factor may be found also in the serum of diabetic animals If I am not mistaken some attempts have been made by Krahl and also by Bornstein to reverse this inhibition with insulin and perhaps Dr Krahl would like to comment on this point since he didn t mention it in his talk The reversibility by insulin of this inhibition would be an important observation

The second paper that of Dr Gaebler had to do with the maintenance of enzymes in tissues under the influence of injected pituitary hormones The reported studies were based on measurements of enzyme levels and here the important question arises—since so many studies are being made on enzyme concentrations—as to how much of a particular enzyme does the cell need to carry out its function We do not know If there is a series of enzymatic reactions one particular enzyme might be regarded as the rate limiting step and its diminution might effect over all processes However other enzymes may be present in great excess and therefore, mask any

and co-factors Under no circumstances have we obtained any effects of insulin We feel that this system should be sensitive to insulin if the hormone is involved in any of the reactions of glucose catabolism

B A HOUSSAY I would like to direct some questions to Dr Krahl In animals injected with pituitary extracts or growth hormone there is a delay in the disorder of carbohydrate of one or two days before you get diabetic signs During this time is there a rapid development of your antagonist—your lipoprotein? The other question concerns the animal without a pancreas and insulin and the fact that insulin and your principle are probably antagonistic Is there a difference either in time of appearance or in amount between the lipoprotein development in serum of an animal deprived of its pancreas and in the serum of an intact animal?

M E KRAHL Perhaps I might now reply to Dr Cori s question as to whether or not insulin has any effect upon the lipoprotein inhibition of glucose utilization in cell free muscle extracts Bornstein and I have done a number of experiments on this question and the reason I did not report on these studies is that the effects of insulin are not completely uniform In about 25 or 30 per cent of the instances in which inhibition was obtained by small concentrations of lipoprotein the insulin reversed this inhibition In the other instances we were unable to get such an effect We do not know how to get a uniform insulin reversal in this system

In answer to Dr Houssay s question as to the time required for this inhibitor in the lipoprotein fraction to develop I can say it takes some hours In the experiments shown in the Figure from Park and Bornstein s paper where the inhibitor disappeared from the serum of diabetic animals when the corresponding group was hypophysectomized one could restore this inhibitor by injecting growth hormone and cortisone into the hypophysectomized diabetic animal This restoration takes some hours The second question I believe was whether or not there is a difference in the concentration of this inhibitor in the diabetic and normal animal We have not investigated this point in detail but, under the present conditions of our test, we have observed inhibition from lipoprotein obtained from the diabetic serum and little or no inhibition from similar liproprotein prepared from normal serum I suspect this is the result of insulin being present in the same fraction of the normal serum

C N H LONG I would like to ask Dr Park a question which I am sure has occurred to many of you I believe he showed that in the alloxan diabetic rat there was no differential concentration between the intracellular and extracellular glucose Now what are his findings in the hypophysectomized depancreatized animal in which Dr Krahl and his colleagues have not found the inhibitory factor?

important When one works with eviscerated animals—and I know that he has performed experiments without evisceration—but, in the eviscerated animal as used by Levine, it is necessary to inject glucose and insulin at the same time, because insulin alone will kill the animal from hypoglycemia Now this means that you are changing the metabolism of the muscle tremendously, as has been known, for example, from the experiments of Best and others on eviscerated cats In these experiments, the injection of glucose with insulin increased the oxygen uptake of muscle tremendously and if Dr Best is here, I would like to have him comment on his old experiments

I think this is enough comment perhaps to start the general discussion except if I may mention for one final point In recent studies conducted at our laboratory on the problem of permeability, using different methods than the distribution of sugar between blood and tissues we found one cell the ascites tumor cell which takes up sugars very readily and as far as we could determine, is not influenced at all by the addition of insulin It does have a cell membrane it does have a transport mechanism, presumably but that alone is not enough to provide the action of insulin

General Discussion

T LEVITT May I congratulate Drs Krahl Gaebler and Park on three excellent and very helpful papers There is one point where I would like to inquire and it is directed at Dr Gaebler I believe he said that hypophysectomy produced a fall in the alkaline phosphatase and that thyroxin restored the alkaline phosphatase but did not increase the bone weight to normal Now I would like to ask him firstly whether or not he noticed in these experiments the effect of total thyroidectomy on alkaline phosphatase bone weight growth and maturation Secondly does tri iodothyronine reduce the thyroxin effects numerically? Thirdly, does growth hormone manifest the same effects as thyroxin in this instance? If not how do they differ?

O H GAEBLER Unfortunately there are no answers to two of these questions because no experiments were done either with tri iodothyronine or in thyroidectomized animals I mentioned the dose of thyroxin which of course would be important in these results

SIDNEY WEINHOUSE Dr Park s paper in particular has impressed me very much There is accumulating evidence that somehow insulin is involved in cell permeability I might mention some work of Mrs Dunn in our laboratory which in a negative way may be cited in support of this idea of insulin action We have carried out some experiments with whole unfractionated homogenates of muscle heart liver and brain In such preparations properly fortified glucose oxidation is very rapid We have been unable to obtain any effect of insulin in such systems Fortunately we are able to limit glucose oxidation in this system by changing the concentration of substrates

and co-factors Under no circumstances have we obtained any effects of insulin We feel that this system should be sensitive to insulin if the hormone is involved in any of the reactions of glucose catabolism

B A HOUSSAY I would like to direct some questions to Dr Krahl In animals injected with pituitary extracts or growth hormone there is a delay in the disorder of carbohydrate of one or two days before you get diabetic signs During this time, is there a rapid development of your antagonist—your lipoprotein? The other question concerns the animal without a pancreas and insulin and the fact that insulin and your principle are probably antagonistic Is there a difference either in time of appearance or in amount between the lipoprotein development in serum of an animal deprived of its pancreas and in the serum of an intact animal?

M E KRAHL Perhaps I might now reply to Dr Cori s question as to whether or not insulin has any effect upon the lipoprotein inhibition of glucose utilization in cell free muscle extracts Bornstein and I have done a number of experiments on this question and the reason I did not report on these studies is that the effects of insulin are not completely uniform In about 25 or 30 per cent of the instances in which inhibition was obtained by small concentrations of lipoprotein the insulin reversed this inhibition In the other instances we were unable to get such an effect We do not know how to get a uniform insulin reversal in this system

In answer to Dr Houssay s question as to the time required for this inhibitor in the lipoprotein fraction to develop I can say it takes some hours In the experiments shown in the Figure from Park and Bornstein s paper where the inhibitor disappeared from the serum of diabetic animals when the corresponding group was hypophysectomized one could restore this inhibitor by injecting growth hormone and cortisone into the hypophysectomized diabetic animal This restoration takes some hours The second question I believe was whether or not there is a difference in the concentration of this inhibitor in the diabetic and normal animal We have not investigated this point in detail but under the present conditions of our test we have observed inhibition from lipoprotein obtained from the diabetic serum and little or no inhibition from similar liproprotein prepared from normal serum I suspect this is the result of insulin being present in the same fraction of the normal serum

C N H LONG I would like to ask Dr Park a question which I am sure has occurred to many of you I believe he showed that in the alloxan diabetic rat there was no differential concentration between the intracellular and extracellular glucose Now what are his findings in the hypophysectomized depancreatized animal in which Dr Krahl and his colleagues have not found the inhibitory factor?

P J RANDLE Dr Krahl commented that growth hormone by itself had no influence on the glucose uptake of the normal rat diaphragm This we have been able to confirm in a variety of concentrations of the hormone The growth hormone has been reported by Park and his associates to have an insulin like action on the glucose uptake of the diaphragm of the hypophysectomized rat when the hormone was added *in vitro* We have been able to show recently using very low concentrations of growth hormone and insulin approximately 0 1 μg of each per ml *in vitro* that growth hormone significantly enhances the action of insulin on the glucose uptake of the isolated diaphragm of the normal rat We have obtained also some confirmation of the existence of the lipoprotein inhibitor in the plasma of the alloxan diabetic rat We observed that plasma from the alloxan diabetic rat unlike that from the normal rat does not stimulate the glucose uptake of the isolated diaphragm of the normal rat When the plasma was repeatedly frozen and thawed under the conditions which Bornstein described then the same plasma sample thereafter was capable of stimulating the glucose uptake of the normal rat diaphragm

I would like to ask Dr Krahl what he considers to be the physiological significance of this inhibitor Does he consider it to be related to the diabetogenic action of growth hormone and if so has he isolated it from the plasma of an animal in which growth hormone is normally diabetogenic?

With respect to Dr Park s comments on the rate of transport of glucose across the cell membrane I have a brief remark Dr Park referred to Dr Ross work in which the rate of transport of glucose across the blood aqueous barrier was measured in rabbits Dr Ross has also studied the influence of a crude pituitary extract which was rich in growth hormone and observed that the extract like insulin increased the transfer rate of glucose across the blood aqueous barrier and did not inhibit the action of insulin in doing so

M E KRAHL In respect to the question about the physiological significance of these lipoproteins I really can do nothing but speculate I would like to say however that we have studied the inhibition of glucose uptake only in diaphragm If this occurs in other tissues such as pancreatic islets the persistence of glucose deprivation in such cells may in the long run lead to their degeneration

With respect to the second question as to whether or not the lipoprotein has been obtained from the plasma of an animal with metahypophyseal diabetes I must reply that we have not run the experiment but hope to do it this winter

C R PARK In the first place I might comment on Dr Cori s discussion He referred to the transport process as being perhaps an active process Ordinarily I think we mean by active transport that the substance is being con

centrated across a membrane against a gradient In the case of glucose or the other sugars we have no evidence for such a process In a naive kind of way I like to think that glucose gets across the cell membrane by what I would call some specific process and that in the case of muscle it is a one way process simply because the muscle hexokinase system acts as a very effective trapping agent

We have no data of our own with respect to whether insulin might affect the transport of glucose in the liver This point raises a very interesting physiological problem because if insulin accelerates glucose transport into the liver cell it might also conceivably accelerate transport out and be cause the liver itself produces large amounts of glucose This might lead to some interesting physiological complications depending on the state of the animal Our data certainly does not indicate at all that the action of insulin on muscle is necessarily the only action of insulin and I think we clearly need to study other tissues particularly the liver

Now Dr Cori mentioned the work of Chaikoff and others which indi cated that the glucose space of both the normal and diabetic animal ap proached an extracellular type of space when measured with radioactive glucose I think *from our own data we might anticipate such results simply* because the effect of insulin on glucose transfer although most striking in the diaphragm and the heart is rather small in the gastrocnemius And of course the gastrocnemius is probably representative of the large mass of skeletal muscle If then the effect is small in this large mass of skeletal muscle I doubt whether the net insulin effect on the whole animal would be picked up In all of our experiments it was necessary of course to inject glucose along with the insulin for if we had injected insulin alone we would have failed to find enough glucose in the blood stream to determine any of the ratios

Dr Cori also pointed out and I think I mentioned previously that this action of insulin on transport is perhaps peculiar to the certain tissues muscles in particular which we have studied I suspect that it will be demonstrated also in other tissues Dr Krahl showed a year or so ago that insulin would accelerate the glucose uptake of fat tissue We attempted to demonstrate an increased transport of glucose in fat tissue This turned out to be a difficult technical problem simply because the intracellular space of adipose tissue is so small that we could not get a satisfactory analysis

Dr Long asked whether or not we have performed any studies in the hypophysectomized rat We haven t done such as yet but we certainly would like to

S J FOLLEY (Reading University) To the one mentioned by Dr Cori I think we could add another tissue which shows an active glucose uptake but which is not affected by insulin For instance slices of mammary glands taken from ruminants by contrast to non ruminants actively utilize the

sugar, not for lipogenesis, but, presumably, for oxidation This is apparent because when one uses labeled sugar, it can be detected in the carbon dioxide and, yet, that process is quite unaffected by the addition of insulin to the medium

I would like to address a question to Dr Park He obtained interesting results concerning the transport of galactose into brain tissue Now it is known that galactose is metabolized in brain Has he considered measuring the effect of insulin on the transport of galactose into mammary tissue where of course, there is a very active metabolism of that sugar?

C R PARK We have not attempted to measure galactose transport in mammary tissue I would like to add I think there are a number of tissues insensitive to this insulin effect The red blood cell has been studied quite extensively in this regard It has apparently, a specific transport process for glucose but does not respond to insulin

24

The Influence of Growth Hormone on Blood Insulin and Glucagon Activity

P J Randle
University of Cambridge Cambridge England

Studies of the part played by insulin in the growth and nitrogen retention induced by growth hormone and of the influence of growth hormone upon the histology and insulin content of the pancreas have suggested that pitui tary growth hormone exerts an influence upon the secretion of insulin by the pancreatic islets These studies the subject of several reviews [1,2,3,4] will have been discussed by others at this symposium This presentation will be limited to a review of attempts to assess directly the influence of growth hormone upon the insulin secretion of the pancreatic islets Measurements were made of blood levels of insulin activity in animals injected with growth hormone in human patients suffering from diseases believed to involve dis ordered secretion of pituitary growth hormone and in blood perfusing the isolated rat pancreas *in vitro* under the influence of growth hormone

The Nature of Plasma Insulin Activity At the present time the minute amounts of insulin in blood plasma can be detected and estimated only by biological methods based upon the blood sugar response of suitably pre pared test animals[5,6,7,8,9] or the *in vitro* glucose uptake of the isolated rat diaphragm [10,11,1] It is doubtful whether these methods give a true estimate of the insulin content of blood plasma since blood plasma may well contain in addition to insulin factors which in the bioassay have themselves an insulin like action or which enhance the action of insulin or which oppose the action of insulin Biological estimates of the insulin content of blood plasma necessarily measure the resultant of these various interacting factors and it is therefore preferable to refer to the biologically determined insulin content of blood plasma as the plasma insulin activity "

Secretion of Insulin by the Isolated Perfused Pancreas Anderson and Long attempted to demonstrate a direct action of growth hormone upon the

sugar, not for lipogenesis, but, presumably, for oxidation This is apparent because when one uses labeled sugar, it can be detected in the carbon dioxide and, yet, that process is quite unaffected by the addition of insulin to the medium

I would like to address a question to Dr Park He obtained interesting results concerning the transport of galactose into brain tissue Now it is known that galactose is metabolized in brain Has he considered measuring the effect of insulin on the transport of galactose into mammary tissue where, of course, there is a very active metabolism of that sugar?

C R PARK We have not attempted to measure galactose transport in mammary tissue I would like to add I think there are a number of tissues insensitive to this insulin effect The red blood cell has been studied quite extensively in this regard It has apparently a specific transport process for glucose but does not respond to insulin

ADHA rats When a growth hormone solution was administered to recipient ADHA rats no change in blood sugar level occurred within 30 minutes When however growth hormone was administered to donor ADHA rats and portal vein blood from these animals injected into recipient ADHA rats a transient but significant rise of blood sugar level occurred in the recipient ADHA rats within 30 minutes of the injection this change of blood sugar level was accompanied by depletion of liver glycogen Similarly when portal (pancreatico-duodenal) vein blood from many growth hormone diabetic or predriabetic cats was injected into recipient ADHA rats a significant hyperglycaemia occurred in these animals By contrast femoral vein blood from the same growth hormone injected cats exerted no hyperglycaemic influence when injected into recipient ADHA rats and indeed in some instances a marked but not significant fall of blood sugar level occurred No change in the blood sugar level of recipient ADHA rats followed the injection of portal or femoral vein blood from saline injected cats or ADHA rats

Bornstein Reid and Young concluded from these experiments that the administration of growth hormone to cats and ADHA rats resulted in the appearance of a hyperglycaemic factor in the portal blood of these animals which was capable of promoting breakdown of liver glycogen in ADHA rats The authors concluded that this factor was in all probability glucagon though as Young has remarked positive identification of this factor must await its isolation from the blood [1] There was no evidence in these experiments that growth hormone influenced the rate of insulin secretion by the pancreatic islets of the cat though the presence of a hyperglycaemic factor in many of the plasma samples might well have prevented the detection of insulin by the blood sugar response of ADHA rats

The possibility that glucagon may be released from the pancreatic islets under the influence of growth hormone in acromegaly is suggested by reports that plasma from diabetic acromegalic patients is hyperglycaemic in the ADHA rat [1 1] In these experiments arm vein blood plasma from diabetic acromegalic patients was found to be hyperglycaemic in the ADHA rat whereas Bornstein Reid and Young found only the portal vein blood of growth hormone injected cats and ADHA rats to be hyperglycaemic in the ADHA rat [13]

Studies with Cross Circulation Experiments Foa and his associates studied the influence of growth hormone upon the insulin and glucagon activity of blood from the pancreatic and mesenteric veins of normal dogs by means of crossed circulation [14] In these experiments two types of crossed circulation were established by connecting the pancreatic or the mesenteric vein of a donor dog to the femoral vein of a recipient dog With dogs connected in this way the intravenous administration of growth hormone to a donor dog was followed by an immediate rise of blood sugar level in both donor and recipient dogs when the cross circulation was of the pancreatic

insulin secretion of the pancreatic islets by means of the isolated perfused pancreas of normal fasted rats[6] Using for the detection of insulin activity in blood the fall in blood sugar level of adrenodemedullated alloxan diabetic hypophysectomised rats (AADH rats) these authors observed that the addition of growth hormone to blood perfusing the isolated pancreas *in vitro* suppressed the secretion of insulin which, otherwise, occurred when the perfusing blood glucose level was high When the glucose level of the perfusing blood was low (within limits of normal, fasting blood glucose level) no secretion of insulin could be demonstrated with or without the addition of growth hormone to the perfusing blood Since growth hormone itself did not influence the detection of insulin by the blood sugar response of AADH rats Anderson and Long concluded that the direct action of growth hormone upon the pancreatic islets was to suppress the secretion of insulin which otherwise occurred in response to hyperglycaemia and that excessive insulin secretion was unlikely to accompany a diabetogenic action of the anterior pituitary The validity of such an inference is made doubtful by more recent work which suggests that glucagon may be released from the pancreatic islets under the influence of growth hormone [13,14] As Young has suggested [1] the release of glucagon into the blood under the influence of growth hormone might well prevent the detection of insulin by a method based on the fall in blood sugar level of AADH rats

Moreover recent studies of the action of growth hormone, administered *in vivo* upon the glucose uptake of the isolated rat diaphragm *in vitro* have shown that the initial action of growth hormone is to stimulate the glucose uptake of the isolated rat diaphragm and that the diabetogenic action of growth hormone (which includes a depression of glucose uptake by the isolated diaphragm of the rat) is not manifested until several hours after the injection of growth hormone [15] It has been suggested further that during this latent period growth hormone is transformed *in vivo* to another molecule with diabetogenic properties[15] (see also [16]) and that this transformation is dependent upon adrenocortical secretion [15,17,18,19] The apparent suppression of insulin secretion by growth hormone observed by Anderson and Long may therefore have been the consequence of an insulin like action of growth hormone upon the pancreatic islets The action of the postulated transformation product of growth hormone upon insulin secretion by the pancreatic islets may be entirely different and the possible importance of the adrenal cortex in this connection must be remembered

Influence of Growth Hormone on Insulin and Glucagon Activity of Blood from Living Animals

Studies with the ADHA Rat Assay for Blood Insulin Bornstein Reid and Young[13] used the blood sugar response of alloxan diabetic hypophysectomised adrenalectomised rats (ADHA rats)[8] to study the insulin activity of blood from saline or growth hormone injected cats and from

ADHA rats When a growth hormone solution was administered to recipient ADHA rats no change in blood sugar level occurred within 30 minutes When however growth hormone was administered to donor ADHA rats and portal vein blood from these animals injected into recipient ADHA rats, a transient but significant rise of blood sugar level occurred in the re cipient ADHA rats within 30 minutes of the injection this change of blood sugar level was accompanied by depletion of liver glycogen Similarly when portal (pancreatico duodenal) vein blood from many growth hormone diabetic or prediabetic cats was injected into recipient ADHA rats a sig nificant hyperglycaemia occurred in these animals By contrast femoral vein blood from the same growth hormone injected cats exerted no hyper glycaemic influence when injected into recipient ADHA rats and indeed in some instances a marked but not significant fall of blood sugar level occurred No change in the blood sugar level of recipient ADHA rats fol lowed the injection of portal or femoral vein blood from saline injected cats or ADHA rats

Bornstein Reid and Young concluded from these experiments that the administration of growth hormone to cats and ADHA rats resulted in the appearance of a hyperglycaemic factor in the portal blood of these animals which was capable of promoting breakdown of liver glycogen in ADHA rats The authors concluded that this factor was in all probability glucagon though as Young has remarked positive identification of this factor must await its isolation from the blood [1] There was no evidence in these experi ments that growth hormone influenced the rate of insulin secretion by the pancreatic islets of the cat though the presence of a hyperglycaemic factor in many of the plasma samples might well have prevented the detection of insulin by the blood sugar response of ADHA rats

The possibility that glucagon may be released from the pancreatic islets under the influence of growth hormone in acromegaly is suggested by re ports that plasma from diabetic acromegalic patients is hyperglycaemic in the ADHA rat [0 1] In these experiments arm vein blood plasma from dia betic acromegalic patients was found to be hyperglycaemic in the ADHA rat whereas Bornstein Reid and Young found only the portal vein blood of growth hormone injected cats and ADHA rats to be hyperglycaemic in the ADHA rat [13]

Studies with ***Cross Circulation Experiments*** *Foa and his associates* studied the influence of growth hormone upon the insulin and glucagon activity of blood from the pancreatic and mesenteric veins of normal dogs by means of crossed circulation [14] In these experiments two types of crossed circulation were established by connecting the pancreatic or the mesenteric vein of a donor dog to the femoral vein of a recipient dog With dogs con nected in this way the intravenous administration of growth hormone to a donor dog was followed by an immediate rise of blood sugar level in both donor and recipient dogs when the cross circulation was of the pancreatic

insulin secretion of the pancreatic islets by means of the isolated perfused pancreas of normal fasted rats[6] Using for the detection of insulin activity in blood the fall in blood sugar level of adrenodemedullated alloxan diabetic hypophysectomised rats (AADH rats) these authors observed that the addition of growth hormone to blood perfusing the isolated pancreas *in vitro* suppressed the secretion of insulin which, otherwise occurred when the perfusing blood glucose level was high When the glucose level of the perfusing blood was low (within limits of normal fasting blood glucose level) no secretion of insulin could be demonstrated with or without the addition of growth hormone to the perfusing blood Since growth hormone itself did not influence the detection of insulin by the blood sugar response of AADH rats Anderson and Long concluded that the direct action of growth hormone upon the pancreatic islets was to suppress the secretion of insulin which otherwise occurred in response to hyperglycaemia and that excessive insulin secretion was unlikely to accompany a diabetogenic action of the anterior pituitary The validity of such an inference is made doubtful by more recent work which suggests that glucagon may be released from the pancreatic islets under the influence of growth hormone[13 14] As Young has suggested[1] the release of glucagon into the blood under the influence of growth hormone might well prevent the detection of insulin by a method based on the fall in blood sugar level of AADH rats

Moreover recent studies of the action of growth hormone administered *in vivo* upon the glucose uptake of the isolated rat diaphragm *in vitro* have shown that the initial action of growth hormone is to stimulate the glucose uptake of the isolated rat diaphragm and that the diabetogenic action of growth hormone (which includes a depression of glucose uptake by the isolated diaphragm of the rat) is not manifested until several hours after the injection of growth hormone[1] It has been suggested further that during this latent period growth hormone is transformed *in vivo* to another molecule with diabetogenic properties[15] (see also [16]) and that this transformation is dependent upon adrenocortical secretion[1 17 18 19] The apparent suppression of insulin secretion by growth hormone observed by Anderson and Long may therefore have been the consequence of an insulin like action *of growth hormone upon the pancreatic islets* The action of the postulated transformation product of growth hormone upon insulin secretion by the pancreatic islets may be entirely different and the possible importance of the adrenal cortex in this connection must be remembered

Influence of Growth Hormone on Insulin and Glucagon Activity of Blood from Living Animals

Studies with the ADHA Rat Assay for Blood Insulin Bornstein Reid and Young[13] used the blood sugar response of alloxan-diabetic hypophysectomised adrenalectomised rats (ADHA rats)[8] to study the insulin activity of blood from saline or growth hormone injected cats and from

ADHA rats When a growth hormone solution was administered to recipient ADHA rats no change in blood sugar level occurred within 30 minutes When however growth hormone was administered to donor ADHA rats and portal vein blood from these animals injected into recipient ADHA rats a transient but significant rise of blood sugar level occurred in the re cipient ADHA rats within 30 minutes of the injection this change of blood sugar level was accompanied by depletion of liver glycogen Similarly when portal (pancreatico duodenal) vein blood from many growth hormone diabetic or prediabetic cats was injected into recipient ADHA rats a sig nificant hyperglycaemia occurred in these animals By contrast femoral vein blood from the same growth hormone injected cats exerted no hyper glycaemic influence when injected into recipient ADHA rats and indeed in some instances a marked but not significant fall of blood sugar level occurred No change in the blood sugar level of recipient ADHA rats fol lowed the injection of portal or femoral vein blood from saline injected cats or ADHA rats

Bornstein Reid and Young concluded from these experiments that the administration of growth hormone to cats and ADHA rats resulted in the appearance of a hyperglycaemic factor in the portal blood of these animals which was capable of promoting breakdown of liver glycogen in ADHA rats The authors concluded that this factor was in all probability glucagon though as Young has remarked positive identification of this factor must await its isolation from the blood [1] There was no evidence in these experi ments that growth hormone influenced the rate of insulin secretion by the pancreatic islets of the cat though the presence of a hyperglycaemic factor in many of the plasma samples might well have prevented the detection of insulin by the blood sugar response of ADHA rats

The possibility that glucagon may be released from the pancreatic islets under the influence of growth hormone in acromegaly is suggested by re ports that plasma from diabetic acromegalic patients is hyperglycaemic in the ADHA rat [0, 1] In these experiments arm vein blood plasma from dia betic acromegalic patients was found to be hyperglycaemic in the ADHA rat whereas Bornstein Reid and Young found only the portal vein blood of growth hormone injected cats and ADHA rats to be hyperglycaemic in the ADHA rat [13]

Studies with Cross Circulation Experiments Foa and his associates studied the influence of growth hormone upon the insulin and glucagon activity of blood from the pancreatic and mesenteric veins of normal dogs by means of crossed circulation [14] In these experiments two types of crossed circulation were established by connecting the pancreatic or the mesenteric vein of a donor dog to the femoral vein of a recipient dog With dogs con nected in this way the intravenous administration of growth hormone to a donor dog was followed by an immediate rise of blood sugar level in both donor and recipient dogs when the cross circulation was of the pancreatic

femoral type With the mesenteric femoral type of cross circulation, the administration of growth hormone to a donor dog was again followed by an immediate rise of blood sugar level in the donor dog but little or no change of blood sugar level occurred in the recipient dog under these conditions These results were not modified when growth hormone was administered to the donor dog over a period of some days before establishment of the cross circulation

Foa and his associates concluded from these experiments that growth hormone promoted the release of a hyperglycaemic factor from the pancreas of the donor dog and that this was probably glucagon There was no evidence in these experiments that growth hormone exerted any influence upon the rate of insulin secretion by the pancreatic islets of the donor dog although Foa and his associates had previously reported that insulin secreted by the pancreatic islets of the donor dog in response to intravenous glucose could be detected in this way This type of experiment does not exclude a possible influence of growth hormone upon insulin secretion by the pancreatic islets of the dog since the release of a hyperglycaemic factor by the pancreas of the donor dog under the influence of growth hormone might well prevent the detection of insulin by a method based on the blood sugar response of the recipient dog

Studies with the Rat Diaphragm Assay for Insulin in Blood Plasma

Thus estimations of insulin activity in blood by methods of insulin assay based on the blood sugar response of AADH or ADHA rats or of intact dogs have provided no definite evidence for an influence of growth hormone upon the secretion of insulin by the pancreatic islets The detection of insulin in blood by such methods might well be prevented by glucagon liberated from the pancreatic islets under the influence of growth hormone[2] Glucagon is capable of neutralising the hypoglycaemic action of insulin[3, 4] and may invalidate, under certain conditions insulin assays based on the hypoglycaemic action of insulin[5]

Groen and his associates[10] reported that the glucose uptake of the isolated rat diaphragm could be used to detect and estimate insulin in blood plasma Young[1] suggested that this technique might be used to detect insulin in blood plasma in the presence of glucagon since glucagon does not oppose the action of insulin upon the glucose uptake of the isolated rat diaphragm[6, 27] The insulin activity of blood from growth hormone injected cats and rats and of blood from patients suffering from acromegaly and hypopituitarism has been re investigated by a method of insulin assay based upon the *in vitro* glucose uptake of the isolated rat diaphragm[1, 4, 3]

Methods and Procedure

Assay of Plasma Insulin Activity by the Isolated Rat Diaphragm

The glucose uptake of the isolated diaphragm of the normal rat is significantly increased by the addition of insulin to the fluid in which the diaphragm is suspended[30] The relationship between the concentration of insulin added *in vitro* to the incubation medium and the glucose uptake of the isolated diaphragm has been explored and the results analysed statistically The experimental procedure which has been rigorously standardised has been described fully elsewhere[1]

Table 1

THE EFFECT OF VARIOUS CONCENTRATIONS OF INSULIN ADDED *In Vitro* UPON THE GLUCOSE UPTAKE OF THE ISOLATED RAT DIAPHRAGM

Insulin Concentration Microunits/ml (= 10⁻⁶ unit)	Absolute Glucose Uptake mg glucose/g of wet diaphragm/hr Mean ± S E of Mean	∛(Absolute Glucose Uptake × 100)		Number of Hemi diaphragms
		Observed	Calculated from Linear Regression	
0	2 32 ± 0 05	—	—	16
125	2 96 ± 0 09	6 665	6 679	16
500	3 55 ± 0 08	7 081	7 090	16
2000	4 25 ± 0 06	7 518	7 505	16
8000	5 03 ± 0 09	7 952	7 910	16
32000	5 73 ± 0 10	8 307	8 321	16

All glucose uptakes with insulin were significantly greater than the basal glucose uptake Each change of insulin concentration resulted in a significant increment in response

The relationship between insulin concentration in the incubation medium and the glucose uptake of the isolated rat diaphragm is illustrated by the results of typical experiments recorded in Table 1 and Figure 1 Over the range of insulin concentration 0 125–32 milliunits insulin/ml there was a linear relation between $\sqrt[3]{}$ (glucose uptake by the diaphragm) and log concentration of insulin added *in vitro* to the incubation medium The results of four determinations of slope standard deviation and index of precision at different times are given in Table 2

A single standard method was used to assay plasma insulin activity[12] In typical assays four hemidiaphragms were used to determine the basal glucose uptake (glucose uptake in the presence of buffer only) and five or six hemidiaphragms to determine the glucose uptake in the presence of plasma (25% plasma in buffer) and of a standard dose of insulin (2 or 4 milliunits/ml) The significance of any increase in the glucose uptake of

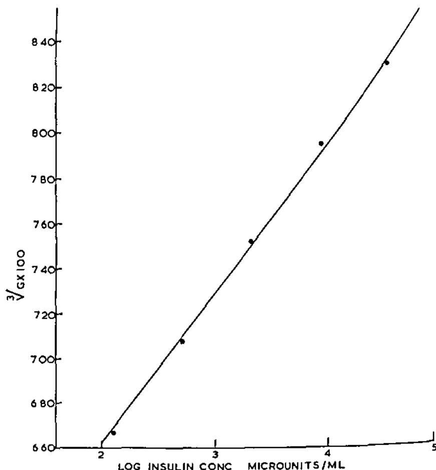


FIG 1 The glucose uptake of the isolated rat diaphragm in the presence of different concentrations of insulin added *in vitro* expressed as a log dose (of insulin) response curve Data derived in September 1953 and recorded in Table 1 (cf see Table 2)

the isolated diaphragm resulting from the presence of insulin or plasma in the incubation medium was established by the *t* test[31 3] (for P = 0 05) the insulin activity of the plasma was then calculated from a log dose (of insulin) response curve previously determined the standard dose of insulin incorporated in the assay being used to fix the position of the log dose response curve In this calculation the slope of the log dose response curve was assumed to be constant the constancy of slope is illustrated by the data in Table 2 The limits of error (P = 0 05) for each estimate of plasma insulin activity were derived from previously determined regression data for crystalline insulin since the variance in different assays has not differed significantly The insulin activity of the plasma was calculated as milliunits insulin/ml of plasma (1 milliunit = 10^{-3} unit)

Table 2

SLOPE OF LOG DOSE RESPONSE CURVE AND INDEX OF PRECISION FOR RAT DIAPHRAGM ASSAY FOR INSULIN

Date	*Slope (b) ± S E of Slope*	*Standard Deviation about Regression Line(s)*	*Index of Precision λ = s b*	*No of Dose Levels*	*Total Number of Hemi diaphragms*
Sept 1953	0 6925 ± 0 033	0 25	0 36	5	80
Jan 1954	0 7760 ± 0 095	0 279	0 36	3	36
April 1954	0 7345 ± 0 056	0 259	0 35	4	48
Sept 1954	0 8281 ± 0 091	0 32	0 38	4	36

The adequacy of this technique has been checked by carrying out replicate assays at different times on many samples of plasma Representative results of many such assays are given in Table 3 These results suggest that *plasma insulin activity* may be assayed confidently as *insulin* under these conditions

Table 3

REPLICATE ASSAYS OF PLASMA INSULIN ACTIVITY BY THE ISOLATED RAT DIAPHRAGM

Nature of Plasma	*Calculated Plasma Insulin Activity Milliunits insulin ml of plasma*	*Limits of Error for P = 0 05*	*N*
Acromegalic	61	24–162	10
(freeze dried plasma)	45	18–144	10
	24	9–62	10
Acromegalic	110	42–284	10
(frozen plasma)	163	63–421	10
Normal Child	22	8–57	12
(free dried plasma)	14	6–36	11
Hypopituitarism	0 77	0 2–2 6	10
(plasma stored at 2 C)	1 00	0 4–2 9	10

N is total number of hemidiaphragms used to determine mean glucose uptake in the presence of insulin and of plasma

Growth Hormone Growth Hormone was prepared from ox pituitary by a modification of the method of Wilhelmi Fishman and Russell[33] Each batch of growth hormone is designated by a preceding number (e g 47GH 48GH) and the three fractions prepared in each batch by a following number (e g 47GH1 47GH2 47GH3) The growth hormone was dissolved in 1 per cent saline containing 1 10 000 merthiolate as preservative and the solutions stored at about 2° C

Experimental Animals RATS Female albino Wistar rats of 150–200 g were used as plasma donors The animals were fed unlimited amounts of rat

cake[34] and water, and growth hormone or saline administered by intraperitoneal injection Blood was collected from the inferior vena cava under Evipan® anaesthesia (10 mg /100 g by intraperitoneal injection) received into a syringe containing a little heparin and the plasma separated immediately by centrifugation The animals were taken from food and the last injection of saline or growth hormone administered 80 minutes before the collection of blood

Hypophysectomised rats were prepared by the usual technique and fed the same diet as normal rats Completeness of hypophysectomy was established by the failure of the animals to gain weight and by post mortem examination of the pituitary fossa

Alloxan diabetic rats were prepared by the intravenous administration of a freshly prepared solution of anhydrous alloxan (dose—50 mg /kg)

CATS Healthy adult male or castrate cats were used as plasma donors The animals were fed an unlimited amount of a meat diet[35] and water, and growth hormone or saline administered by subcutaneous injection into the neck Supplementary feeds of milk and fish were given to animals showing anorexia during growth hormone treatment Blood was collected from the exposed femoral vein under Evipan® anaesthesia (50–80 mg /kg by intraperitoneal injection) received into a syringe containing a little heparin and the plasma separated by centrifugation The animals were taken from food two hours before blood was collected and the last injection of growth hormone or saline given 80 minutes before blood was collected (except in two experiments where the interval was 24 hours) Certain cats were used as plasma donors on more than one occasion and in such cases an interval of at least two weeks rest was allowed between the two experiments

Normal cats are defined as animals not previously injected with growth hormone who had a plasma sugar of less than 150 mg /100 ml (i e a blood sugar level of about 100 mg /100 ml) at the time of blood collection *Growth hormone diabetic cats* are defined as animals who developed glycosuria in response to growth hormone injection which ceased after the injections were discontinued and who had a plasma sugar greater than 150 mg /100 ml at the time of blood collection *Growth hormone prediabetic cats* are defined as animals who had received a number of daily growth hormone injections and who had a plasma sugar of less than 150 mg /100 ml at the time of blood collection

Pancreatectomy was carried out under combined Evipan® and ether anaesthesia Postoperatively the animals were given penicillin for six days and fed a meat diet[3] with supplements of fish and raw pancreas The diabetes was controlled by daily injections of soluble insulin at 10 00 A M and 6 00 P M The animals were not used as plasma donors for at least ten days after operation by which time the diabetes was controlled by a constant daily amount of insulin Growth hormone was administered and blood collected as for normal cats the last injection of insulin being given 80 minutes

before the collection of blood Completeness of pancreatectomy was estab lished at post mortem by naked eye examination of the operation area

Results

The insulin activity of plasma from saline injected normal rats under these conditions was equivalent to 11 milliunits insulin/ml of plasma (limits of error 7–19 milliunits/ml) The injection of growth hormone in amounts sufficient to produce significant extra growth resulted in no change in plasma insulin activity (mean plasma insulin activity equivalent to 13 milliunits insulin/ml plasma limits of error 8–21 milliunits/ml) The indi vidual results and details of growth hormone treatment are recorded in Figure 2

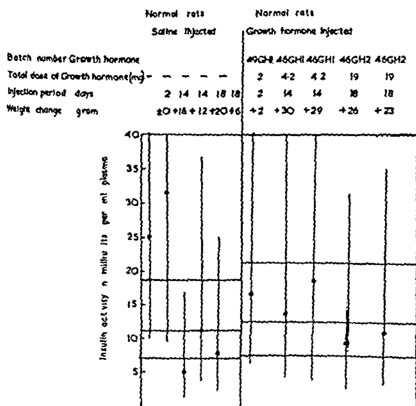


FIG 2 The insulin activity of plasma from saline injected and growth hor mone injected normal rats estimated by the glucose uptake of the isolated rat diaphragm Each dot represents the observed plasma insulin activity and the extremes of the vertical lines the limits of error for $P = 0\cdot05$ The horizontal lines represent the mean plasma insulin activity for each group of animals and the limits of error for $P = 0\cdot05$

Hypophysectomy consistently resulted in a marked and significant reduction of plasma insulin activity and in the majority of hypophysectomised rats no insulin activity could be detected in the plasma (Figs 3 and 4) The injection of growth hormone into hypophysectomised rats was followed in some instances by restoration of plasma insulin activity to normal levels provided the growth hormone injections were begun immediately after operation and blood was collected within 4 or 5 days of hypophysectomy With longer periods of injection or in experiments in which animals were kept for 7 to 10 days after hypophysectomy before the injections of growth hormone were begun growth hormone failed to exert any significant influence upon the plasma insulin activity even though significant growth occurred under these conditions The individual results and details of growth hormone treatment are given in Figure 4

The insulin activity of plasma from saline injected normal cats was equivalent to 4 4 milliunits insulin/ml plasma (limits of error 3 4–5 7 milliunits/ml) The insulin activity of plasma from growth hormone injected prediabetic or diabetic cats was significantly greater than the insulin activity

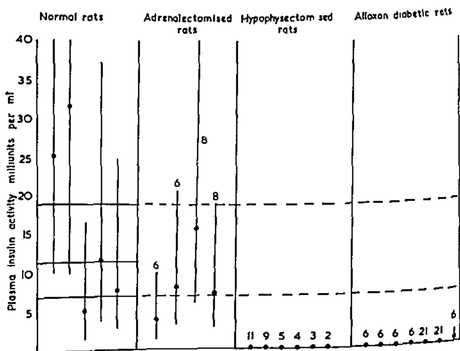


FIG 3 The insulin activity of plasma from normal alloxan diabetic adrenalectomised and hypophysectomised rats estimated by the glucose uptake of the isolated rat diaphragm Figures with each estimate of plasma insulin activity refer to number of days after adrenalectomy hypophysectomy or alloxan administration

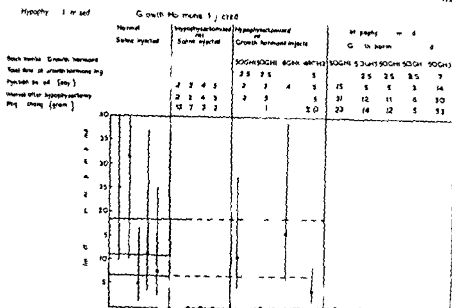


FIG 4 The insulin activity of plasma from saline and growth hormone injected hypophysectomised rats estimated by the glucose uptake of the isolated rat diaphragm

of plasma from saline injected normal cats the range of values being from 11 6 milliunits/ml to 164 milliunits ml (mean = 49 milliunits ml) The individual results and details of growth hormone treatment are recorded in Figure 5 In two experiments blood was collected from saline injected normal cats before and five minutes after the intravenous administration of 2 5 mg of growth hormone Under these conditions growth hormone administration resulted in no change in plasma insulin activity (Fig 5)

The insulin activity of plasma from depancreatised cats treated with insulin was equivalent to 7 4 milliunits insulin/ml plasma (limits of error 5 5–9 8 milliunits/ml) This is not significantly different from the insulin activity of plasma from saline injected normal cats When growth hormone was administered to depancreatised cats receiving a constant amount of insulin no change in the insulin activity of the plasma occurred though the administration of similar amounts of growth hormone to intact cats was consistently followed by a significant increase in plasma insulin activity When however growth hormone was administered to insulin injected depancreatised cats and the dose of insulin increased during the period of growth hormone administration a very considerable and highly significant increase in plasma insulin activity occurred (Fig 6)

The insulin activity of plasma from normal people was equivalent to 11 5 milliunits insulin/ml (limits of error 8 3–15 8 milliunits/ml) The insulin

	NORMAL					PRE DIABETIC			DIABETIC						
Cat n mber	277	267	274	273	269	269	273	274	271	274	267	194	271	273	279
G owth hormon Batch Number						SOGH2	SOGH3	SOGH3	SOGH3	SOGH2	SOGH1	SOGH3	SOGH1	SOGH2	S3GH1
Total dose of Growth hormone (mg)						30	30	75	30	45	75	60	35	35	55
I ject n per od (days)						5	6	8	6	8	8	6	7	7	7
Pla ma s ga mg per 100ml	74	116	112	110	120	150	120	116	360	350	260	301	360	262	274
L t inj ct on (hours)						13	13	13	13	13	13	13	24	24	13
Du ation of Diabetes (days)									0	0	0	3	2	2	0

Pl ma insulin a tivity milliunit insul per ml plasma: 1000, 100, 10, 1

log pla ma in lin activity: 3, 2, 1, 0

Fig 5 The insulin activity of plasma from saline injected cats and from growth hormone injected prediabetic and diabetic cats estimated by the glucose uptake of the isolated rat diaphragm

Open circles in first panel (normal cats) represent estimates of insulin activity of plasma collected five minutes after the intravenous administration of 2 5 mg growth hormone The estimates immediately preceding the open circles refer to the insulin activity of plasma collected immediately before growth hormone administration Estimates of plasma insulin activity in pairs (panels for prediabet c and d abetic cats) refer to the insulin activity of fresh plasma (est mate on the left) and the in ul n activity of the same pla ma ample after peated f ng and thawing (estimate on the right)

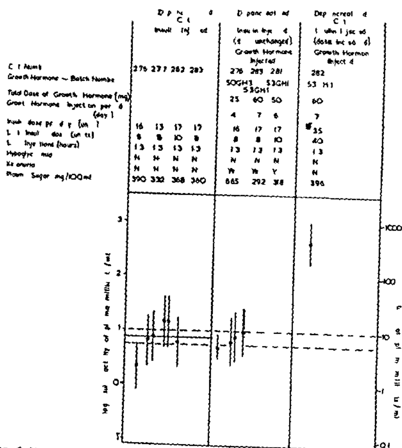


FIG 6 The insulin activity of plasma from depancreatised cats injected with insulin and with insulin + growth hormone estimated by the glucose uptake of the isolated rat diaphragm For explanation of paired estimates of plasma insulin activity see legend of Table 5

Insulin dose refers to dose on day before blood collected

activity of plasma from many cases of acromegaly was significantly greater than the insulin activity of plasma from normal people those cases of acro megaly with normal plasma insulin activity were considered on clinical grounds to be inactive Three diabetic acromegalic patients showed ele vated plasma insulin activity two of these cases had mild diabetes not re quiring insulin treatment and the third case which was severely diabetic was well controlled by 80 units of lente insulin each day at the time of the investigation Two cases of pituitary gigantism were also studied and both showed considerably elevated plasma insulin activity By contrast the in sulin activity of plasma from several cases of hypopituitarism was signifi cantly less than the insulin activity of plasma from normal people The individual results are recorded in Figure 7

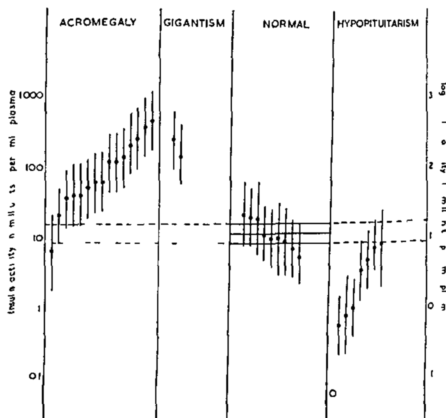


FIG 7 The insulin activity of plasma from normal people acromegalic patients and hypopituitary patients estimated by the glucose uptake of the isolated, rat diaphragm Each estimate of plasma insulin activity refers to an individual patient ○ This plasma sample was without demonstrable insulin activity

One case of acromegaly showed a marked reduction of plasma insulin activity following pituitary irradiation (plasma insulin activity before irradiation 60 milliunits/ml plasma insulin activity three months after pituitary irradiation 5 milliunits/ml) A similar reduction of plasma insulin activity followed pituitary irradiation in a pituitary giant (plasma insulin activity before pituitary irradiation 244 milliunits/ml plasma insulin activity three months after pituitary irradiation 18 milliunits/ml)

The enhanced insulin activity of acromegalic plasma is emphasized by experiments in which the influence of acromegalic and normal human plasma upon the glucose uptake and glycogen synthesis of the isolated rat diaphragm were studied simultaneously Acromegalic plasma promoted significantly greater glucose utilisation and glycogen synthesis by isolated rat hemi diaphragms than did normal human plasma (Table 4)

Table 4

INFLUENCE OF NORMAL HUMAN AND ACROMEGALIC PLASMA UPON THE GLUCOSE UPTAKE AND GLYCOGEN SYNTHESIS OF THE ISOLATED RAT DIAPHRAGM

Sample No	*Plasma Collected from*	*Blood Sugar mg per 100 ml*	*Glucose Uptake mg glucose per g of wet diaphragm per hour Mean ± SE of Mean*	*Glycogen Synthesis mg glycogen glucose per g of wet diaphragm per hour Mean ± SE of Mean*	*No of Hemi diaphragms*
1a	Normal adult person	70	2.84 ± 0.15	—	4
1b	Diabetic patient receiving insulin	305	2.86 ± 0.21	—	4
1c	Acromegalic diabetic receiving insulin	360	4.00 ± 0.21	—	4
	t for *1a 1b* versus *1c* is 4.10 whence P<0.01				
2a	No plasma—buffer only	—	1.67 ± 0.16	0.428 ± 0.022	8
2b	Normal person	80	2.60 ± 0.13	0.731 ± 0.049	8
2c	Acromegalic diabetic receiving insulin	190	1.62 ± 0.09	1.350 ± 0.054	8

Glucose *t* for *2a* versus *2b* is 4.65 whence P<0.001
t for *2a* versus *2c* is 11.1 whence P<0.001
t for *2b* versus *2c* is 6.74 whence P<0.001

Glycogen *t* for *2a* versus *2b* is 6.00 whence P<0.001
t for *2a* versus *2c* is 18.9 whence P<0.001
t for *2b* versus *2c* is 9.1 whence P<0.001

Interpretation of Results

Specificity of the Rat Diaphragm Assay for Insulin Activity in Plasma These results confirm earlier reports that the glucose uptake of the isolated rat diaphragm may be used to detect insulin activity in plasma[10 11 1 17 19 35] The extent to which such insulin activity may be attributed to insulin in the plasma is by no means certain Normal human plasma, like insulin increases both the glucose uptake and the glycogen synthesis of the isolated rat diaphragm (Table 4)[10 36] and this action of plasma like that of insulin is abolished by treatment with cysteine[10 11 1] The plasma of alloxan diabetic rats (Figure 3)[17 19 36] depancreatised dogs[10] and severely diabetic patients[10 7] possesses considerably less insulin activity than plasma from normal animals of the same species such observations would appear to support the view that the insulin like action of plasma upon the glucose uptake of rat diaphragm can be attributed to insulin in the plasma[10 11] However Park and Bornstein have isolated from the plasma of alloxan diabetic rats an endocrine factor which inhibits the glucose uptake of the isolated, rat diaphragm and have attributed the diminished insulin activity

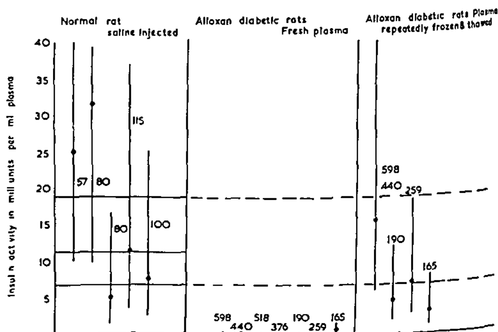


FIG 8 The influence of repeated freezing and thawing upon the insulin activity of plasma from alloxan diabetic rats estimated by the glucose uptake of the isolated rat diaphragm Figures refer to plasma sugars in mg /100 ml Two figures together (last panel) indicate that the assay was carried out on a sample derived by mixing in equal proportion two plasma samples with these sugar contents Plasma was alternately frozen and thawed in solid CO_2/acetone mixture and water at 37 C

of alloxan diabetic rat plasma to the presence of this inhibitor and not to a reduced insulin content of the plasma [17 18 19] Bornstein relating the inhibitory action of the plasma to the β_1 lipoprotein fraction reported that this activity of plasma was abolished by repeated freezing and thawing [19] Some evidence in support of these observations of Park and Bornstein has been obtained in experiments in which the insulin activity of fresh alloxan diabetic rat plasma was compared with the insulin activity of the same plasma subjected to repeated freezing and thawing In confirmation of earlier reports by other workers [17 18 30] fresh plasma from alloxan diabetic rats possessed considerably less insulin activity than plasma from normal rats (Fig 8) After repeated freezing and thawing the same plasma samples possessed significantly greater insulin activity which was less than but not significantly different from that of normal rat plasma (Fig 8) These experiments support the claim of Park and Bornstein that the diminished insulin like action of plasma from alloxan diabetic rats upon the glucose uptake of rat diaphragm must result at least in part from the presence of an inhibitor in the plasma which is inactivated by freezing and thawing and not merely from the diminished insulin content of alloxan diabetic rat plasma It may well be that the diminished insulin like action of plasma from depancreatised dogs and severely diabetic patients upon the glucose uptake of the isolated rat diaphragm results at least in part from the presence of an inhibitor in the plasma and not merely from its reduced insulin content

Park and Bornstein concluded from their observations[17 19] that the insulin like action of normal rat plasma upon the glucose uptake of the isolated rat diaphragm was due to a non specific effect of plasma protein It has not been possible to confirm this contention since (a) plasma from many cases of human hypopituitarism was significantly less effective than normal human plasma in stimulating the glucose uptake of the isolated rat diaphragm (Fig 7) and (b) the plasma of hypophysectomised and alloxan diabetic hypophysectomised adrenalectomised rats (ADHA rats[17]) has no influence upon the glucose uptake of the isolated rat diaphragm An inhibitor of the type described by Park and Bornstein cannot be responsible for the diminished insulin activity of plasma from hypopituitary patients and hypophysectomised and ADHA rats since the presence of this inhibitor in the plasma is dependent upon both pituitary and adrenal secretions [19]

The insulin like action of plasma from normal animals upon the glucose uptake of the isolated rat diaphragm would appear to depend upon the presence of at least three endocrine factors in the plasma

(a) Insulin since the insulin activity of normal human plasma is completely and consistently abolished by treatment with cysteine and insulin is the only substance inactivated by cysteine which is known to be capable of stimulating the glucose uptake of the isolated normal rat diaphragm

(b) Growth hormone or some factor whose presence is conditioned by growth hormone since the insulin activity of plasma from acutely hypophysectomised rats can be restored to normal levels by treatment with growth hormone (Fig 4)

(c) Some pituitary or pituitary controlled factor other than growth hormone since the insulin activity of plasma from the chronic hypophysectomised rat is not restored to normal levels by treatment with growth hormone (Fig 4)

The extent to which each of these factors contributes to the insulin activity of plasma from normal animals and the nature of the unidentified pituitary or pituitary controlled factor have yet to be determined

The Insulin Activity of Plasma in Relation to Growth Hormone The enhanced insulin activity of plasma from growth hormone injected intact cats and from acromegalic patients might result from (a) any insulin like action of the extra growth hormone in the plasma[37] (but see also [15]) or (b) an insulin like action of some other factor possibly insulin itself secreted in response to growth hormone Extra growth hormone is unlikely by itself to be responsible for the enhanced insulin activity of the plasma since (a) growth hormone has no significant effect upon the glucose uptake of the isolated normal rat diaphragm under these conditions (Table 5 see

Table 5

THE INFLUENCE OF PITUITARY GROWTH HORMONE ADDED *in vitro* UPON THE GLUCOSE UPTAKE OF THE ISOLATED RAT DIAPHRAGM INCUBATED IN BUFFER

Concentration of Growth Hormone Added in vitro μg/ml	*Glucose Utilization mg glucose/g of wet diaphragm/hour Mean ± SE of Mean*	*Number of Hemidiaphragms*	*Comment*
None	2 25 ± 0 14	8	No significant difference between any of these means
0 1	2 46 ± 0 24	8	
1	2 24 ± 0 21	8	
10	2 27 ± 0 22	8	
50	2 34 ± 0 25	8	
100	2 58 ± 0 24	8	

also [15]) (b) growth hormone does not enhance the insulin activity of normal human plasma when added to the plasma *in vitro* (Table 6) and (c) a single intravenous injection of growth hormone has no influence upon the insulin activity of plasma collected from normal cats five minutes after the injection (Fig 5) The enhanced insulin activity of plasma from growth hormone injected intact cats and from acromegalic patients must depend therefore upon a change in the plasma level of some factor whose presence is conditioned by growth hormone The possibility that this factor is *insulin* is strongly suggested by the failure of growth hormone injection to enhance the insulin activity of plasma from insulin treated depancreatised cats un

Table 6

INFLUENCE OF PITUITARY GROWTH HORMONE ADDED *in Vitro* UPON THE INSULIN LIKE ACTION OF NORMAL HUMAN PLASMA UPON THE GLUCOSE UPTAKE OF THE ISOLATED RAT DIAPHRAGM

Normal Human Plasma Sample Number	*Concentration of Growth Hormone Added in Vitro μg /ml*	*Batch Number of Growth Hormone*	*Glucose Uptake mg glucose/g of wet diaphragm/hr Mean ± SE of Mean*	*No of Hemi diaphragms*	*Comment*
1	None 0 1 10 100	48GH1	3 13 ± 0 37 3 02 ± 0 17 3 11 ± 0 23 3 14 ± 0 16	6 6 6 6	
2	None 0 1 10 100	50GH1	3 17 ± 0 15 3 27 ± 0 19 3 39 ± 0 16 3 3[illegible] ± 0 13	6 6 6 6	No significant difference be tween any of these means
3	None 0 1 1 10	51GH1	[illegible] 62 ± 0 16 [illegible] 58 ± 0 21 2 86 ± 0 13 2 77 ± 0 17	6 6 6 6	

(b) Growth hormone or some factor whose presence is conditioned by growth hormone since the insulin activity of plasma from acutely hypophysectomised rats can be restored to normal levels by treatment with growth hormone (Fig 4)

(c) Some pituitary or pituitary controlled factor other than growth hormone *since the insulin activity of plasma from the chronic hypophysectomised* rat is not restored to normal levels by treatment with growth hormone (Fig 4)

The extent to which each of these factors contributes to the insulin activity of plasma from normal animals and the nature of the unidentified pituitary or pituitary controlled factor have yet to be determined

The Insulin Activity of Plasma in Relation to Growth Hormone The enhanced insulin activity of plasma from growth hormone injected intact cats and from acromegalic patients might result from (a) any insulin like action of the extra growth hormone in the plasma[3] (but see also [15]) or (b) an insulin like action of some other factor possibly insulin itself secreted in response to growth hormone Extra growth hormone is unlikely by itself to be responsible for the enhanced insulin activity of the plasma since (a) growth hormone has no significant effect upon the glucose uptake of the isolated normal rat diaphragm under these conditions (Table 5 see

Table 5

THE INFLUENCE OF PITUITARY GROWTH HORMONE ADDED *in Vitro* UPON THE GLUCOSE UPTAKE OF THE ISOLATED RAT DIAPHRAGM INCUBATED IN BUFFER

Concentration of Growth Hormone Added in vitro μg /ml	*Glucose Utilisation mg glucose/g of wet diaphragm/hour Mean ± SE of Mean*	*Number of Hemidiaphragms*	*Comment*
None	2 25 ± 0 14	8	No significant difference between any of these means
0 1	2 46 ± 0 24	8	
1	2 24 ± 0 21	8	
10	2 27 ± 0 22	8	
50	2 34 ± 0 25	8	
100	2 58 ± 0 24	8	

also [15]) (b) growth hormone does not enhance the insulin activity of normal human plasma when added to the plasma *in vitro* (Table 6) and (c) a single intravenous injection of growth hormone has no influence upon the insulin activity of plasma collected from normal cats five minutes after the injection (Fig 5) The enhanced insulin activity of plasma from growth hormone injected intact cats and from acromegalic patients must depend therefore upon a change in the plasma level of some factor whose presence is conditioned by growth hormone The possibility that this factor is *insulin is strongly* suggested by the failure of growth hormone injection to enhance the insulin activity of plasma from insulin treated depancreatised cats un

growth hormone may be transformed *in vivo* to another form capable of inhibiting the glucose utilisation of muscle since it has yet to be conclusively demonstrated that growth hormone, itself is capable of such an action[15] (and also Tables 5 6 and 7) Evidence in support of this hypothesis has recently been obtained by Park and Bornstein[17 18 19] who have separated from the plasma of alloxan diabetic hypophysectomised rats (ADH rats) injected with growth hormone *and* cortisone a lipoprotein factor capable of inhibiting the glucose uptake of the isolated normal rat diaphragm *in vitro* This lipoprotein inhibitor was not found in the plasma of ADH rats injected with either growth hormone or cortisone alone and its action could not be reproduced *in vitro* by adding growth hormone and cortisone to the fluid in which the isolated diaphragm was suspended This inhibitor was reported to be inactivated by repeated freezing and thawing [19]

The presence of such an inhibitor in the plasma of growth hormone injected intact or depancreatised cats might well diminish the insulin activity of such plasma samples as estimated by the glucose uptake of the isolated rat diaphragm No evidence for the presence of such an inhibitor in the plasma of growth hormone injected intact or depancreatised cats was obtained in experiments in which the insulin activity of fresh plasma from these animals was compared with the insulin activity of the same plasma samples after repeated freezing and thawing (Figs 5 and 6)

General Discussion

Insulin Secretion in Relation to the Growth and Diabetogenic Actions of Growth Hormone

Studies of the nitrogen retained by intact depancreatised and depancreatised hypophysectomised cats under the influence of growth hormone led Milman de Moor and Lukens to conclude that growth hormone promotes the secretion of extra insulin by the pancreatic islets of the intact cat [38] Gaebler and Robinson obtained evidence for a similar influence of crude growth hormone upon the pancreatic islets of the dog [39] Estimations of plasma insulin activity by means of the glucose uptake of the isolated rat diaphragm support the concept that growth hormone promotes directly or indirectly an enhanced rate of insulin secretion by the pancreatic islets of the intact cat and suggest also that a temporary form of diabetes may appear as a consequence of growth hormone injection at a time when the blood level of insulin is still elevated These observations would also be consonant with the view that the permanent form of diabetes which may appear in intact cats (and dogs) as a consequence of growth hormone injection is to be attributed to the deficient insulin secretion of pancreatic islets damaged by the increased demands for insulin in the earlier phases of growth hormone diabetes [40] It is possible that permanent diabetes in acromegaly may arise in like fashion as a consequence of the sustained

less the dose of insulin is increased during the period of growth hormone injection (Fig 6) The amount of extra insulin required to be present in the plasma from growth hormone injected intact cats and from acromegalic patients to account for the enhanced insulin activity of some of these plasma samples would have to be as much as 0 1 unit/ml of plasma and it seems unlikely that the pancreatic islets could maintain such a high plasma insulin level Growth hormone can enhance however, the action of insulin *upon the glucose uptake of the isolated, normal rat diaphragm* when both hormones are added together *in vitro* to the suspending fluid (Table 7) and the presence of *extra* insulin and *extra* growth hormone in plasma from growth hormone injected intact cats and from acromegalic patients might well account for the enhanced insulin activity of these plasma samples The final proof of such a possibility must await the isolation of those factors responsible for the insulin activity of plasma from acromegalic patients and growth hormone injected intact cats

Table 7

INFLUENCE OF PITUITARY GROWTH HORMONE AND INSULIN ADDED SEPARATELY AND TOGETHER *in Vitro* UPON THE GLUCOSE UPTAKE OF THE ISOLATED RAT DIAPHRAGM INCUBATED IN BUFFER

Incubation Medium	*Glucose Utilization mg glucose/g of wet diaphragm/hour Mean ± SE of Mean*	*No of Hemi diaphragms*	*Comment*
Buffer	2 32 ± 0 08	23	
Buffer + growth hormone –concentration 0 1μg /ml	2 30 ± 0 05	23	*t* for *a* versus *b* is 11 6 P<0 001
Buffer + insulin –concentration 0 1μg /ml	3 27 ± 0 10	23	*t* for *a* versus *c* is 12 7 P<0 001
Buffer + insulin + growth hormone in above concentrations	3 80 ± 0 08	23	*t* for *b* versus *c* is 4 27 P<0 001

With this proviso in mind these experiments support the view that growth hormone can provoke an enhanced rate of insulin secretion by the pancreatic islets of intact cats and acromegalic patients but not of the intact rat But it must be emphasized that still lacking is a direct and unequivocal demonstration that growth hormone stimulates the secretion of insulin by the islets through a direct or an indirect mechanism

Inhibitors in Plasma in Relation to Growth Hormone Reid Smith and Young[16] and, more recently Park and his associates[15] have emphasized that

The possibility that glucagon secreted in response to growth hormone may contribute to the diabetogenic action of growth hormone is suggested by the ability of glucagon to raise the blood sugar level of intact cats dogs and rabbits[47 48 49] and to antagonise the hypoglycaemic action of insulin in the rabbit [3 4] Such a possibility is not excluded by the action of growth hormone in intensifying the glycosuria of depancreatised cats and dogs[58 59] and depancreatised hypophysectomised cats[59] since it seems probable that tissues other than the pancreas contain glucagon and they may well be capable of secreting it [61]

Acknowledgements

I wish to thank the Medical Research Council for generous financial aid in support of my work on plasma insulin activity and Professor F G Young for much helpful advice and criticism during the course of this work and the preparation of this paper

References

1 Young F G *Recent Progress in Hormone Research* 8 471 (1953)
2 Lukens F D W *Experimental Diabetes* Eds Delafresnaye J F and G Howard Smith Oxford Basil Blackwell & Mott Ltd 1954 263
3 de Jongh S E *Experimental Diabetes* Eds Delafresnaye J F and G Howard Smith Oxford Basil Blackwell & Mott Ltd 1954 242
4 Haist R E *Physiol Revs* 24 409 (1944)
5 Anderson E Lindner E and V Sutton *Am J Physiol* 149 350 (1947)
6 Anderson E and J A Long *Recent Progress in Hormone Research* 2 209 (1948)
7 Anderson E and J A Long *Endocrinology* 40 98 (1947)
8 Bornstein J *Australian J Exp Biol Med Sci* 28 87 (1950)
9 Yenerman M Cornfield J Bates R W and E Anderson *Federation Proc* 12 162 (1953)
10 Groen J Kamminga C E Willebrands A F and J R Blickman *J Clin Invest* 31 97 (1952)
11 Vallance Owen J and B Hurlock *Lancet* 1 68 (1954)
12 Randle P J *Brit Med J* 1 1237 (1954)
13 Bornstein J Reid E and F G Young *Nature* (London) 168 903 (1951)
14 Foa P P Magid E B Glassman M D and H R Weinstein *Proc Soc Exp Biol Med* 83 758 (1953)
15 Park C R Brown D H Cornblath M Daughaday W H and M E Krahl *J Biol Chem* 197 151 (1952)
16 Reid E Smith R H and F G Young *Biochem J* 42 xix (1948)
17 Park C R *Phosphorus Metabolism* Eds McElroy W D and B Glass Baltimore John Hopkins Press II 634 (1952)
18 Bornstein J and C R Park *J Biol Chem* 205 503 (1953)
19 Bornstein J *J Biol Chem* 205 513 (1953)
20 Bornstein J and P Trewhella *Med J Australia* 1 119 (1951)
21 Bornstein J and R D Lawrence *Brit Med J* 2 1541 (1951)
22 Foa P P Weinstein H R and J A Smith *Am J Physiol* 157 197 (1949)

hypersecretion of growth hormone believed to occur in all active cases of this disorder since plasma from acromegalic patients shows enhanced insulin activity when estimated by the glucose uptake of the isolated rat diaphragm Proof of such possibilities must await the estimation of blood insulin levels in cats (and dogs) rendered permanently diabetic by treatment with growth hormone

The intact rat unlike the adult cat does not become diabetic when injected with growth hormone but merely exhibits an accelerated rate of growth Young[41] suggested that this might be the result of (a) the ability of the pancreatic islets of the rat to sustain a greatly accelerated rate of insulin secretion or (b) different activity or reactivity of enzymes in growing animals such that growth hormone can promote growth in the rat without increasing the demands of the tissues for insulin Scott and Engel[8] using indirect criteria could find no evidence for an enhanced rate of insulin secretion by the pancreatic islets of rats injected with growth hormone Estimations of blood insulin activity by means of the blood sugar response of AADH rats[6] and of plasma insulin activity by means of the glucose uptake of the isolated, rat diaphragm have suggested that growth hormone does *not* promote an enhanced rate of insulin secretion by the pancreatic islets of the rat It may well be the unlimited ability to grow in response to growth hormone which protects the rat from the diabetogenic action of growth hormone Such a possibility was envisaged by Engel and his associates[43] who observed that growth hormone is diabetogenic in adrenocorticotropin injected force fed rats since adrenocorticotropin is able to antagonise the growth promoting action of growth hormone in the rat[43,44]

It would be unwise to draw any general conclusions as to the influence of growth hormone upon insulin secretion from studies of plasma insulin activity in three animal species in relation to growth hormone but it may be that growth hormone only promotes an enhanced rate of insulin secretion by the pancreatic islets of those animals in which it is diabetogenic Proof of such a possibility must await the estimation of blood insulin levels by more specific methods in many animal species and in animals of the same species at different ages

Glucagon Secretion in Relation to the Growth and Diabetogenic Actions of Growth Hormone

The observations of Bornstein et al[13] and those of Foa et al[14] referred to in an earlier section, suggest that growth hormone can provoke the secretion of glucagon by the pancreatic islets of the cat rat and dog It has yet to be shown conclusively that glucagon secreted in this way contributes to the growth promoting action of growth hormone in the rat for reports that glucagon like growth hormone is capable of increasing the width of the tibial epiphysis when administered to hypophysectomised rats[46] could not be confirmed by other workers[47]

25

The Influence of Growth Hormone and Other Factors on the Islets of Langerhans and the Pancreas

R E Haist
Department of Physiology University of Toronto

The pituitary gland under some circumstances may exert effects on both the internal and external secreting parts of the pancreas Four consequences of removal of the pituitary gland will be indicated namely the effect on the total mass of the islets of Langerhans the effect on the pancreas as a whole the effect on certain pancreatic enzymes and the effect on a pancreas stimulating hormone of the intestine secretin In addition the influence of growth hormone on the resultant changes will be examined

When the pituitary gland is removed the endocrine pancreas unlike the adrenals thyroid and gonads shows very little atrophy Removal of the pituitary prevents the islets of Langerhans from growing but the total mass of the islets is not much reduced The pancreas as a whole however shrinks in size This results in a *relative* increase in islet tissue so the concentration of islet tissue in the pancreas is greatly increased These points are illustrated in Figure 1 which compares islet weights estimated by a slight modification of the method of Haist and Pugh [1] in hypophysectomized and paired fed intact rats

When crude saline extracts of bovine anterior pituitary gland are injected into hypophysectomized rats islet growth is again obtained This has been demonstrated by Richardson and Young[3] and others Similarly with injections of highly purified growth hormone preparations there is an increase in islet weight in hypophysectomized animals [4] as indicated in Figure 2 This increase is proportional to the increase in body weight hence the islet weight per unit of body weight is not greatly changed However the increase in the size of the pancreas as a whole is such that the *concentration* of islet tissue in the pancreas is actually reduced by growth hormone administration

23 de Duve C Hers H G and J B Bouchaert *Arch intern pharmacodynamie* **72** 45 (1945)
24 Tyberghein J *Arch intern physiol* **60** 105 (1952)
25 Root M A Ellis J and A Staub *Proc Soc Exp Biol Med* **85** 507 (1954)
26 Smith R H cited by Young F G *Ciba Foundation Colloquia on Endocrinology* Ed G E W Wolstenholme London Churchill VI 241 (1953)
27 Randle P J Unpublished data
28 Randle P J *Lancet* **1** 441 (1954)
29 Randle P J *Lancet* **1** 809 (1954)
30 Gemmill C L *Johns Hopkins Hosp Bull* **66** 232 (1940)
31 Chambers E G *Statistical Calculation* C U P 42 (1952)
32 Fisher R and F Yates *Statistical Tables* 2nd ed London Oliver and Boyd Ltd 1943 30
33 Wilhelmi A E Fishman W H and J A Russell *J Biol Chem* **176** 735 (1948)
34 Parkes A S *J Hyg* **44** 491 (1946)
35 Young F G *Biochem J* **39** 515 (1945)
36 Tuerkischer E and E Wertheimer *Biochem J* **42** 603 (1948)
37 Ottaway J H *Brit Med J* **2** 357 (1953)
38 Milman A E De Moor P and F D W Lukens *Am J Physiol* **140** 98 (1951)
39 Gaebler O H and A R Robinson *Endocrinology* **30** 627 (1942)
40 Campbell J Munroe J S Hausler H R and I F W Davidson *Experimental Diabetes* Eds Delafresnaye J F and G Howard Smith Oxford Basil Blackwell & Mott Ltd 217
41 Young F G *Brit Med J* **2** 1167 (1951)
42 Scott J L Jr and F L Engel *Endocrinology* **46** 582 (1950)
43 Engel F L Viau A Coggins W and W S Lynn Jr *Endocrinology* **50** 100 (1952)
44 Li C H *Harvey Lectures Ser* **46** 181 (1950–1951)
45 Li C H *Ciba Foundation Colloquia on Endocrinology* Ed G E W Wolstenholme London Churchill **V** 115 (1953)
46 Elrick H *Proc Soc Exp Biol Med* **82** 76 (1953)
47 Geschwind I I and A Staub *Proc Soc Exp Biol Med* **84** 244 (1953)
48 Staub A Sinn L and O K Behrens *Science* **117** 628 (1953)
49 Foa P P Santamaria L Berger S Smith J A and H R Weinstein *Proc Soc Exp Biol Med* **80** 635 (1952)
50 Campbell J Hausler H R Munroe J S and I W F Davidson *Endocrinology* **53** 549 (1953)
51 Sutherland E W Cori C F Haynes R and N S Olsen *J Biol Chem* **180** 825 (1949)

in hypophysectomized rats In the intact animal (Fig 3) growth hormone administration leads to significant increases in islet weight concentration of islet tissue in the pancreas and islet weight per unit of body weight Thus we see that as far as the endocrine pancreas is concerned injections of purified growth hormone preparations stimulate its growth in hypophysectomized and intact rats This finding differs somewhat from that reported by Abrams Baker Ingle and Li [5] but is consonant with much other information In the hypophysectomized rat the growth stimulating effect on the islets is proportional to the effect on body weight but in the intact rat the

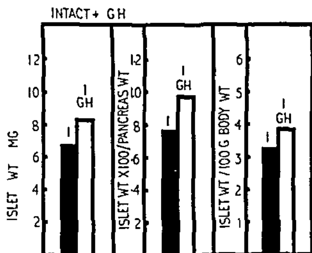


FIG 3 The effect on islet weight of daily subcutaneous injections of highly purified growth hormone preparations for 21–28 days in intact rats
I—intact controls fed *ad libitum*
IGH—intact rats injected with growth hormone fed *ad libitum*

islet increase is relatively greater than the increase in body weight This difference probably results from the more dramatic effect of these growth hormone preparations on body growth in hypophysectomized as compared to intact animals Because growth hormone preparations stimulate the islets to increase in amount it does not necessarily follow that these materials directly influence the islets Though a direct effect is not excluded the effect is more likely an indirect one Other pituitary preparations also stimulate islet growth For example adrenocorticotropin when given in large enough amounts or by continuous intravenous infusion will cause an increase in the total islet weight in intact and in hypophysectomized rats A similar effect is obtained with cortisone [6] ACTH when given by continuous intravenous infusion at the rate of 5 I U for 7 days in intact rats resulted in a very dramatic increase in islet weight an increase much greater than that obtained with the daily subcutaneous injection of the same amount of ACTH for a longer period This emphasizes the fact that in addition to

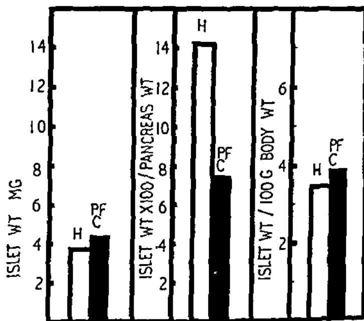


Fig 1 The effect of hypophysectomy on the islet weight H—hypophysectomized PFC—paired fed intact controls

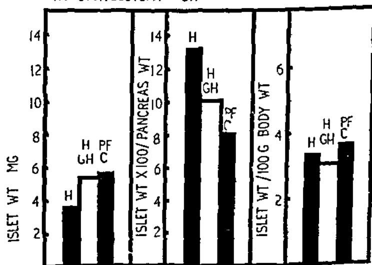


Fig 2 The effect on islet weight of daily subcutaneous injections of highly purified growth hormone preparations (1 mg daily) for 21–28 days in hypophysectomized rats

H—hypophysectomized
HGH—hypophysectomized injected with growth hormone
PFC—intact controls paired fed with HGH

While it has been demonstrated that there is an increase in islet mass following the administration of highly purified growth hormone preparations these experiments do not indicate the type of cell involved in this increase Certainly in the dog and cat in which growth hormone preparations are diabetogenic there is good evidence that the beta cells of the islets are profoundly influenced by growth hormone preparations In connection with this work in the rat however differential islet cell counts have not been made as yet and the influence on specific cell types has not been determined

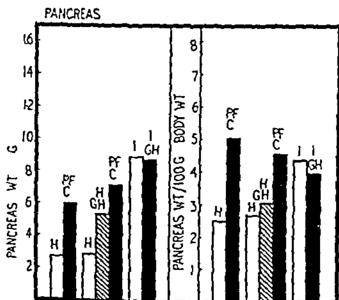


Fig 4 The effect of hypophysectomy and of highly purified growth hormone preparations on pancreas weights
H—hypophysectomized PFC—paired fed controls
HGH—hypophysectomized injected with growth hormone I—intact
IGH—intact, injected with growth hormone preparations

Dr Baker showed very beautifully in a previous meeting some influences of the pituitary on gastric and salivary glands and he presented as well information concerning some pituitary effects on the pancreas In the past we have emphasized as others before us have pointed out that following removal of the pituitary gland the islet changes are not as dramatic as the changes in the pancreas as a whole (Fig 4) Not only is the absolute weight of the pancreas greatly decreased but also it is reduced in relation to body weight The injection of growth hormone preparations into hypophysectomized animals restores the pancreas to a large extent though not completely The weight of the pancreas increases to a proportionately greater extent than does the body weight but this difference is slight and the

considering the dose required for a particular response the method of administration is of great importance also The administration over a period of time of prolactin of thyroid materials or of certain gonadal substances as indicated by Dr Houssay and his associates[7] also will lead to islet hyperplasia and an increase in islet weight[8] Thus the effect is not specific for growth hormone and some final common mediation is probably indicated As far as the islets are concerned those factors stimulating secretion also stimulate growth Since the administration of a number of these varied substances will lead to an islet increase in the absence of the pituitary gland no single pituitary material can be considered as the final common mediator However most of the factors which occasion the islet increase also raise the level of blood sugar or enhance the requirements for insulin The continuous intravenous infusion of glucose has been shown to result in an increase in islet tissue[9] and Dr Lukens and his group have observed that when glucose was infused locally into a part of the pancreas the systemic blood sugar level was reduced and hyperplasia of the islets occurred[10] It is conceivable then that the final common mediation may be through an increase in the level of sugar in the blood or through a reduction in the level of insulin in the blood or both

Table 1

THE EFFECT ON THE ISLETS OF LANGERHANS OF THE ADMINISTRATION OF GROWTH HORMONE PREPARATION AND OF GROWTH HORMONE PLUS GLUCOSE

Group	*No Rats*	*Mean Final Body Wt g*	*Mean Islet Wt mg*
Saline	6	242	9 2
G H	13	261	11 5
Glucose + G H	6	255	14 2

One further experiment of interest in relation to the influence of growth hormone on the islets was carried out by Mr Kinash in our laboratory When growth hormone was continuously infused intravenously for seven days at the rate of 1 mg growth hormone per rat per day there was an increase in the islet weight as compared to saline infused controls (Table 1) This increase was rather similar in extent to that obtained with the continuous infusion of glucose When glucose was infused continuously and given simultaneously to the growth hormone preparation which was administered daily by subcutaneous injection the increase in islet weight was greater than with glucose or growth hormone alone This finding is against the view that growth hormone has an inhibitory influence on the stimulating effect of an elevated blood glucose level on the islets of Langerhans

considered in relation to the body weight or in relation to the total nitrogen of the pancreas The administration of crude saline anterior pituitary extract daily for 4 weeks gave some increase in amylase activity in hypophysectomized animals but injections of growth hormone preparations did not lead to an increase in the total amylolytic activity of the pancreas despite the fact that they caused the pancreas weight to be increased Indeed because of this increase in pancreatic weight the concentration of amylolytic activity in the pancreas was reduced [1]

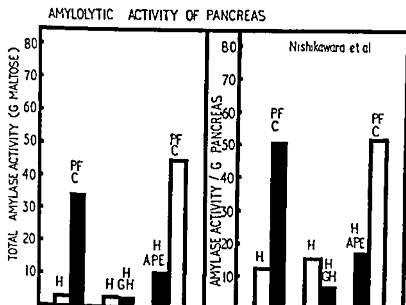


FIG 6 The effect on the amylolytic activity of the pancreas of hypophysectomy and of injections of growth hormone and anterior pituitary extracts in hypophysectomized rats
H—hypophysectomized
PFC—paired fed controls
HGH—hypophysectomized injected with growth hormone
HAPE—hypophysectomized injected with a crude saline extract of the anterior pituitary gland

The best effect in the restoration of the pancreatic amylolytic activity was obtained by the use of thyroid materials Miss M Maykut and later Dr Nishikawara demonstrated that the administration of desiccated thyroid by mouth to hypophysectomized rats led to a large increase in the amylolytic activity of the pancreas restoring it almost to normal[13] (Fig 7) When both thyroid and anterior pituitary extract were given together the total amount of amylolytic activity was not much different from that obtained with thyroid alone but the increase in the weight of the pancreas was sufficiently large to give a reduction in the concentration of amylolytic activity [1] The

pancreatic weight per unit of body weight is not greatly increased In the intact animal the administration of growth hormone preparations has little effect on the weight of the pancreas and indeed if anything causes it to be slightly reduced Cortisone and adrenocorticotropin when given in sufficiently large doses also enhance the pancreas weights in hypophysectomized rats (Fig 5) but not in intact rats Thyroid administration increases the pancreas weights in both the hypophysectomized and intact animals Since removal of the pituitary causes the pancreas as a whole to be reduced but has little effect on the islets it may be concluded that this procedure leads to a profound reduction in the external secreting part of the pancreas The administration of growth hormone preparations brings this back toward normal again but does not completely restore it

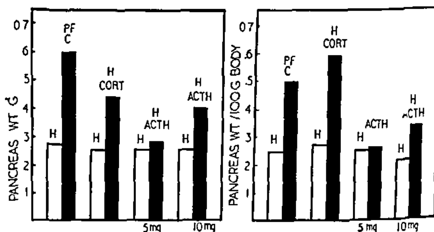


FIG 5 The effect of daily subcutaneous injections of cortisone and of ACTH on the weight of the pancreas in hypophysectomized rats The cortisone dosage was 5 mg daily for 28 days and the ACTH dosages were 5 mg daily and 10 mg daily

H—hypophysectomized PFC—paired fed controls H CORT—hypophysectomized injected with cortisone H ACTH—hypophysectomized injected with ACTH

Because there was such a large change in the external secreting portion of the pancreas in hypophysectomized animals it seemed of some interest to find what changes might occur in the concentration and total amount of certain of the digestive enzymes of the pancreas Some information concerning the amylase activity of the pancreas expressed as grams of maltose is shown in Figure 6 It will be evident that there is a profound reduction in *amylolytic activity* as a result of removal of the pituitary gland This was first shown by Dr J Barrett in our laboratory and has been confirmed by Miss M Maykut and Dr Margaret Nishikawara [11] This reduction in the total amylolytic activity of the pancreas is associated with a lower *concentration* of amylolytic activity and the amylase activity is also reduced when

that removal of the pituitary gland led to a reduction in the total amount and in the concentration of secretin activity in the small intestine (Fig 8)[14] Crude saline extracts of the anterior pituitary gland and growth hormone preparations restored to a large extent the secretin levels of the gut in hypophysectomized rats It was found that adrenocorticotropin also restored the secretin levels The administration of anterior pituitary extract to intact animals occasioned an increase in the secretin levels whereas the administration of growth hormone preparations to intact rats did not It is interesting in this connection that thyroid administration which did restore the pancreas weights and amylolytic activity of the pancreas to a large extent had little or no influence on the secretin levels of the gut and as you have seen growth hormone preparations which restore the secretin levels of the gut had no influence on the amylolytic activity of the pancreas It would appear then that the changes in amylolytic activity of the pancreas are not directly associated with changes in the secretin levels of the intestine While it may be dangerous to argue concerning changes in the liberation of a hormone on the basis of changes in the level of the hormone in a tissue

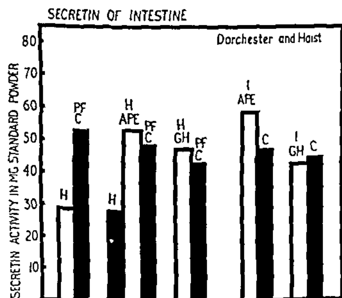


FIG 8 The effect on the secretin activity of the small intestine of hypophysectomy and of injections of anterior pituitary extract and growth hormone preparations in hypophysectomized and intact rats
H—hypophysectomized
PFC—paired fed controls
HAPE—hypophysectomized injected with anterior pituitary extracts
HGH—hypophysectomized injected with growth hormone preparations
IAPE—intact injected with anterior pituitary extracts
IGH—intact injected with growth hormone preparations
C—controls fed *ad libitum*

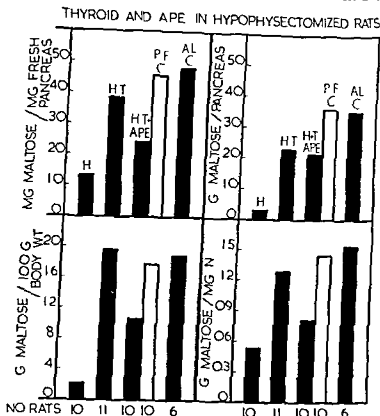


FIG 7 The effect on the amylolytic activity of the pancreas of hypophysectomy and of the administration of desiccated thyroid by mouth to the hypophysectomized rat (Maykut Nishikawara and Haist) The influence of thyroid administration along with daily doses of a crude saline extract of the anterior pituitary gland for 28 days is also indicated (Nishikawara and Haist)

H—hypophysectomized

H + T—hypophysectomized plus thyroid

H + T + APE—hypophysectomized given thyroid and anterior pituitary extract

PFC—paired fed controls

ALC—controls fed *ad libitum*

enzyme studies showed that certain enzymes of the pancreas are not necessarily affected by those factors such as growth hormone which influence the total amount of pancreatic tissue The growth hormone preparations influenced the weight of the pancreas but did not affect the total amylolytic activity

The total proteolytic activity of the pancreas was also reduced after hypophysectomy but the changes in concentration were variable Changes in the pancreas might conceivably be the result of an altered stimulation of the pancreas by hormones of the intestine such as secretin or pancreozymin When the levels of secretin in the intestine were investigated it was found

that removal of the pituitary gland led to a reduction in the total amount and in the concentration of secretin activity in the small intestine (Fig 8) [14] Crude saline extracts of the anterior pituitary gland and growth hormone *preparations restored to a large extent the secretin levels of the gut in* hypophysectomized rats It was found that adrenocorticotropin also restored the secretin levels The administration of anterior pituitary extract to intact animals occasioned an increase in the secretin levels whereas the administration of growth hormone preparations to intact rats did not It is interesting in this connection that thyroid administration which did restore the pancreas weights and amylolytic activity of the pancreas to a large extent had little or no influence on the secretin levels of the gut and as you have seen growth hormone preparations which restore the secretin levels of the gut had no influence on the amylolytic activity of the pancreas It would appear then that the changes in amylolytic activity of the pancreas are not directly associated with changes in the secretin levels of the intestine While it may be dangerous to argue concerning changes in the liberation of a hormone on the basis of changes in the level of the hormone in a tissue

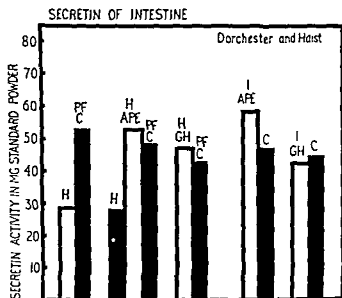


FIG 8 The effect on the secretin activity of the small intestine of hypophysectomy and of injections of anterior pituitary extract and growth hormone preparations in hypophysectomized and intact rats
H—hypophysectomized
PFC—paired fed controls
HAPE—hypophysectomized injected with anterior pituitary extracts
HGH—hypophysectomized injected with growth hormone preparations
IAPE—intact injected with anterior pituitary extracts
IGH—intact injected with growth hormone preparations
C—controls fed *ad libitum*

nevertheless it seems probable that the changes in pancreatic amylolytic activity do not reflect changes in the stimulation of the pancreas by secretin It is possible that there is a better relation between pancreas weights and secretin levels though there are some discrepancies here also Whether or not pancreozymin is involved in this effect is not known as yet There is the possibility, too that changes in the pancreas and in the pancreatic amylolytic activity result from an altered absorption of food materials from the gut The absorption of glucose has been reported to be reduced by hypophysectomy[15] and restored by thyroid administration [16] Conceivably this might affect the amylase activity of the pancreas However the prime reason for the pancreatic changes has not been determined

Summing up we may say that removal of the pituitary gland in the rat leads to little change in the total amount of islet tissue in the pancreas but the pancreas as a whole is greatly reduced in size In addition hypophysectomy is followed by a definite decrease in the pancreatic amylase activity and a diminution in the *secretin* activity of the small intestine Daily injections of highly purified growth hormone preparations increase the islet weight pancreas weight and secretin activity of the intestine but under the conditions of these experiments not the amylase activity of the pancreas In the intact rat similar injections of growth hormone preparations increase the islet weight but neither the weight of the pancreas as a whole nor the secretin level of the intestine were altered

A point of some interest arising from these experiments and evident to an increasing extent in other work also is that the pituitary gland has a profound influence not only on the metabolism of certain substances and on other endocrine structures concerned with this metabolism but also on glands of external secretion important in the digestion and preparation for absorption of these materials

References

1 Haist R E and E J Pugh *Am J Physiol* 152 36 (1948)
2 Bryans F E Kinash B Ashworth M A and R E Haist *Diabetes* 1 358 (1952)
3 Richardson K C and F G Young *J Physiol* 91 352 (1937)
4 Kinash B MacDougall I Evans M A Bryans F E and R E Haist *Diabetes* 2 112 (1953)
5 Abrams G D Baker B L Ingle D J and C H Li *Endocrinology* 53 252 (1953)
6 Kinash B and R E Haist *Am J Physiol* (in press 1954)
7 Houssay B A *Brit Med J* 2 505 (1951)
8 Kerr E H Stears J C MacDougall I and R E Haist *Am J Physiol* 170 448 (1952)
9 Kinash B and R E Haist *Can J Biochem Physiol* 32 428 (1954)
10 Brown E M Dohans F C Freedman L R De Moor P and F D W Lukens *Endocrinology* 50 644 (1952)

11 Nishikawara M Barrett J Maykut M Sprague L and R E Haist *Federation Proc* 13 105 (1954)
12 Nishikawara M and R E Haist Unpublished data
13 Maykut M Nishikawara M and R E Haist Unpublished data
14 Dorchester J E C and R E Haist *J Physiol* 119 266 (1953)
15 Phillips R A and P Robb *Am J Physiol (Proc)* 109 82 (1934)
16 Russell J A *Am J Physiol* 122 547 (1938)

DISCUSSION
Growth Hormone and Cellular Systems

Designated Discussion

LESLIE BENNETT (University of California School of Medicine) During the last hour we have been privileged to hear two fine papers dealing with one of the most vexacious and puzzling questions in the field of anterior pituitary physiology This is the question as to the nature of the control of the structure and function of the islets of Langerhans As pointed out this morning and in previous sessions it is well established that when metahypophyseal or metasomatotropin diabetes is present the islets of Langerhans show structural abnormalities the insulin content of the pancreas is negligible and the ability of the pancreas to secrete insulin into the blood stream may be markedly impaired if not lost Growth hormone produces these changes crude anterior pituitary extracts presumably because of the growth hormone content produce the same changes The questions which have been raised by these and other observations may be restated I believe as follows does growth hormone by some extra pancreatic mechanism elevate blood glucose and then does the elevated blood glucose per se injure the islets of Langerhans and the insulin secreting mechanism? Does growth hormone inhibit insulin secretion directly or does growth hormone inhibit the glucose stimulated secretion of insulin? Does growth hormone stimulate the secretion of insulin directly and thus produce an exhaustion atrophy of the islets? Is it to be regarded as a tropic hormone for the beta cells? If it is a tropic hormone for the beta cells there is no analogy with the other tropic hormones since one cannot produce an exhaustion atrophy of the adrenal cortex for instance by the continuous administration of ACTH Does growth hormone stimulate the secretion of glucagon? If so how does the increased glucagon secretion contribute to the development of the metasomatotropin diabetes? Or how does this stimulation injure the islets of Langerhans?

For some time in Berkeley we have been interested in this field of research and have made certain observations which I think are pertinent to points raised by both of the previous speakers Dr Haist pointed out that growth hormone given to the hypophysectomized rat produces an increase in the mass of the islets Yesterday Professor Houssay commented on the fact that when growth hormone was given to the partially depancreatized

nevertheless it seems probable that the changes in pancreatic amylolytic activity do not reflect changes in the stimulation of the pancreas by secretin It is possible that there is a better relation between pancreas weights and secretin levels, though there are some discrepancies here also Whether or not pancreozymin is involved in this effect is not known as yet There is the possibility too that changes in the pancreas and in the pancreatic amylolytic activity result from an altered absorption of food materials from the gut The absorption of glucose has been reported to be reduced by hypophysectomy[15] and restored by thyroid administration [16] Conceivably this might affect the amylase activity of the pancreas However the prime reason for the pancreatic changes has not been determined

Summing up we may say that removal of the pituitary gland in the rat leads to little change in the total amount of islet tissue in the pancreas but the pancreas as a whole is greatly reduced in size In addition hypophysectomy is followed by a definite decrease in the pancreatic amylase activity and a diminution in the *secretin* activity of the small intestine Daily injections of highly purified growth hormone preparations increase the islet weight pancreas weight and secretin activity of the intestine but under the conditions of these experiments not the amylase activity of the pancreas In the intact rat similar injections of growth hormone preparations increase the islet weight but neither the weight of the pancreas as a whole nor the secretin level of the intestine were altered

A point of some interest arising from these experiments and evident to an increasing extent in other work also is that the pituitary gland has a profound influence not only on the metabolism of certain substances and on other endocrine structures concerned with this metabolism but also on glands of external secretion important in the digestion and preparation for absorption of these materials

References

1 Haist R E and E J Pugh *Am J Physiol* **152** 36 (1948)
2 Bryans F E Kinash B Ashworth M A and R E Haist *Diabetes* **1** 358 (1952)
3 Richardson K C and F G Young *J Physiol* **91** 352 (1937)
4 Kinash B MacDougall I Evans M A Bryans F E and R E Haist *Diabetes* **2** 112 (1953)
5 Abrams G D Baker B L Ingle D J and C H Li *Endocrinology* **53** 252 (1953)
6 Kinash B and R E Haist *Am J Physiol* (in press 1954)
7 Houssay B A *Brit Med J* **2** 505 (1951)
8 Kerr E H Stears J C MacDougall I and R E Haist *Am J Physiol* **170** 448 (1952)
9 Kinash B and R E Haist *Can J Biochem Physiol* **32** 428 (1954)
10 Brown E M Dohans F C Freedman L R De Moor P and F D W Lukens *Endocrinology* **50** 644 (1952)

Haist s comment regarding specific granules or specific cell types since these cells are degranulated and do not stain specifically for either alpha or beta cells When the animals just mentioned are given growth hormone the degree of glycosuria as I said decreases and one sees in some animals at least a rather marked increase in the number of healthy looking islet cells which though large, remain degranulated These changes are shown in Figure 2 I believe we are dealing with the phenomenon mentioned by Professor Houssay yesterday and Dr Haist a few moments ago

We were greatly privileged some years ago when Professor Houssay visited our laboratory in Berkeley at the time of the Herzstein lecture to have him demonstrate to us the technique of pancreatic transplant the procedure which he had used so successfully in his study of the insulin secreting ability of the pancreas of the dog with metahypophyseal diabetes In the last few years, we have used this procedure in an effort to study the acute effects of growth hormone administration on the ability of the pancreas

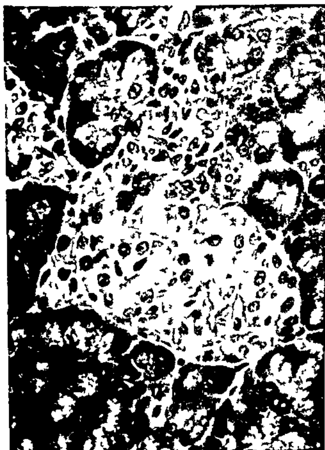


Fig 2

rat hyperplasia and hypertrophy of the islets resulted and the onset of diabetes was prevented Now with many growth hormone preparations and in studies over many years we have observed in diabetic rats only a transient increase in the glycosuria when growth hormone was given These studies have been made in animals with an established glycosuria and it is immaterial whether the rats are diabetic from alloxan or from the effects of a partial pancreatectomy By the 3rd or 4th day of growth hormone treatment the glycosuria usually returns to its normal level and may drop below this level and remain so for as long as we have given the hormone which is a matter of several weeks We likewise have failed to cure diabetes After stopping the hormone the glycosuria returns to essentially the control level When one studies the islets in the pancreatic remnants of these animals one sees certain very interesting changes In Figure 1 is shown an islet from a pancreatic remnant of a diabetic rat and one sees rather marked fibrosis and degenerating very large cells I cannot discuss Dr



FIG 1

age blood sugar values of 5 animals with the transplants The range of variation is shown by the dotted lines Curve *A* is the average of a number of control animals with similar neck dissections and they show no fall in blood sugar during the control experiment If one infuses insulin into these experimental animals one can reproduce the blood sugar changes caused by the normal pancreas only by varying the rate of insulin administration In Figure 4 curve *B* is again the data from the dogs with the transplants The dotted line *curve A is an average curve for 5 animals which received insulin* at varying rates of infusions The insulin dose ranged from 02 to 08 unit per kg per hour We believe then that this is an approximation of the rate at which the normal dog s pancreas is secreting insulin under these circumstances

If one now gives insulin at the rate shown in Figure 4 and just prior to this administers growth hormone one obtains data as shown in Figure 5 These data we believe demonstrate growth hormone antagonism to insulin since the same dose of insulin as used previously is having a reduced hypoglycemic effect Should we now implant a normal pancreas and give

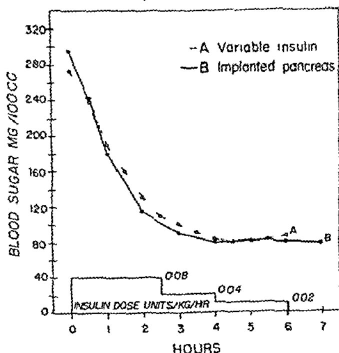


FIG 4

to secrete insulin I believe these observations are pertinent to the data which Dr Randle presented namely that growth hormone given to the cat results apparently in an increase of the blood insulin level In these experiments with pancreatic transplants one uses two dogs i e a donor animal and a recipient From the donor one dissects the duodenum and the attached pancreas preserving the pancreaticoduodenal artery and the pancreaticoduodenal vein These two vessels are then anastomosed by means of cannulae to the common carotid artery and the external jugular vein respectively of the recipients In all the experiments data from which I would like to show the recipient was a totally depancreatized dog which had recovered from the operation The dog was maintained on insulin to such an extent that we achieved a standardized degree of incomplete control with a fasting hyperglycemia of about 300 mg per cent If one then transplants a normal pancreas into such an animal, the blood sugar promptly falls reaches a normal level between 2½ and 3 hours and is maintained at normal values for the succeeding 4 hours In Figure 3 curve *B* represents the aver

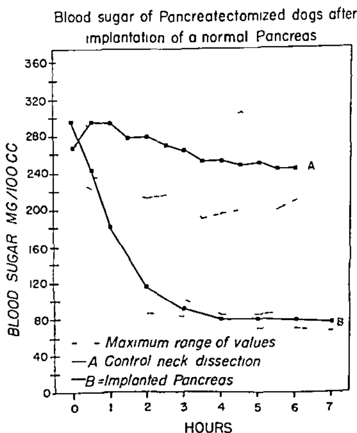


FIG 3

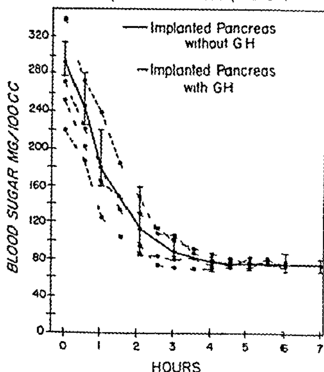


FIG 6

General Discussion

HERBERT SARETT (Mead Johnson Company) I would like to make a brief comment related to Dr Park s paper earlier this morning and also bearing on one point mentioned by Dr Haist We have compared the rate of absorption of glucose and fructose in normal rats and in alloxan diabetic rats and have found that the alloxan diabetic rat absorbed both glucose and fructose much faster than did the normal animals The relative rates of glucose and fructose absorption are the same that is similar to the rate pointed out by Dr Cori in the 1920 s but the absolute rate is increased in both cases

ARNOLD LAZAROW (University of Minnesota) I would like to emphasize that in comparing the cytological studies of islet tissue with the functional secretion of insulin it is important to separate the component elements That is the activities of insulin synthesis insulin storage (presumably as secretion granules) and the release of insulin from the cell into the blood stream may be separately controlled phenomena We don t expect them all to go together necessarily

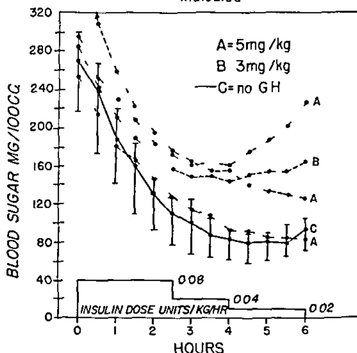


FIG 5

growth hormone there are several possibilities e g if the growth hormone were to inhibit the insulin secretion of the normal pancreas then the resulting blood sugar should be represented by curves which would fall above the dotted curves of Figure 5 If the growth hormone were to have no effect upon insulin secretion but only exerted its anti insulin action then blood sugar values would be about as shown by the dotted curves of Figure 5 If on the other hand the growth hormone were to stimulate insulin secretion the resulting blood sugars would be represented by a family of curves in the region of curve *C* of Figure 5

In Figure 6 are shown the data obtained from such experiments i e the normal pancreas was implanted and growth hormone was given The dotted curves represent blood sugar values from dogs into which was transplanted a normal pancreas and which also received growth hormone These data fit with the last alternative mentioned above We would like to interpret these data as being consistent with an increased insulin secretion from the dog pancreas following the acute administration of growth hormone You will note that there is no evidence here which would lend any support to the view that glucagon is being produced

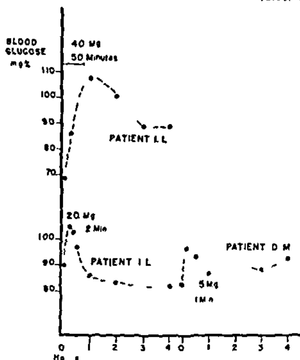

INTRAVENOUS OXYCEL FRACTION IN ADDISON'S DISEASE
BLOOD GLUCOSE mg%
40 Mg
50 Minutes
PATIENT I L
20 Mg
2 Min
PATIENT I L
PATIENT D M
5 Mg
110
100
90
80
70
100
90
80
0
1
2
3
4
0
1
2
3
4

Fig 7

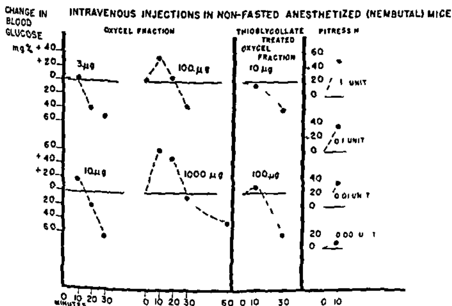

CHANGE IN BLOOD GLUCOSE mg%
INTRAVENOUS INJECTIONS IN NON-FASTED ANESTHETIZED (NEMBUTAL) MICE
OXYCEL FRACTION
THIOGLYCOLLATE TREATED OXYCEL FRACTION
PITRESSIN
3 µg
100 µg
10 µg
1 UNIT
10 µg
1000 µg
100 µg
MINUTES

Fig 8

I would like to ask Dr Randle to say a bit more about the level of insulin which he has reported in blood The values he gives are about 10 milliunits per ml which are several hundred fold greater than what Bornstein and Anderson have reported Furthermore if you were to take the level in man which he reports and assume that insulin has a life of say 24 hours it would be necessary to give a man without islet function about 500 units of insulin a day

P J RANDLE It is quite true, as the previous speaker said that the insulin activity—and that is the term I wish to emphasize—is some 30 times greater than the value which Bornstein obtained I will remind the previous speaker that Hagedorn and associates who extracted insulin from normal human plasma by means of an acid alcohol procedure and used the Anderson rat to assay the resultant extract obtained a value which was 10 times greater than that reported by Bornstein The point I wish to greatly emphasize is that what we are reporting is *insulin activity* What proportion of it represents true insulin is not known This undoubtedly is true for the other methods of assay which have been applied to the estimation of insulin in blood If one turns to some of the acromegalics then the levels expressed as insulin amount to as much as one half unit of insulin per milliliter This is quite ridiculous in all probability for the insulin level of plasma In my presentation I did emphasize that our data are not direct evidence for an influence of growth hormone upon blood insulin levels it is indirect evidence We still await a specific estimate of insulin in plasma

M S RABEN There are several pituitary factors which have an immediate effect on blood sugar and I should like to show three slides of data which Miss Cobb and I have obtained The oxycel purified fraction which Dr Astwood mentioned yesterday as lowering the blood sugar in mice raises the blood sugar in people In Figure 7 are data from three experiments in patients with Addison s disease demonstrating a striking rise in blood sugar which occurred within 10 minutes of the intravenous injection Because the patients complained of symptoms very characteristic of pitressin action when 20 mg of this preparation was rapidly given intravenously we returned to the mouse in which animal we had found this fraction to produce a rapid lowering of blood sugar Now the blood sugar was lowered within one half hour in the mouse but as noted in Figure 8 when 10 minute samples were taken following any dose larger than 3 micrograms the blood sugar rose in 10 minutes and then subsequently fell The larger the dose the more substantial was the rise and actually the rise dampened or delayed the fall if the dose was large enough When the material was treated with thioglycollate the substance that caused the 10 minute rise in the blood sugar seemed to have been destroyed or at least markedly inactivated The material that caused the fall however was not harmed so that in the column

I am more in the mood for letting the audience in on my confusion than I am for trying to explain these observations

F D W LUKENS It has been a great pleasure to listen to a session of the symposium that ends up in far greater harmony than was achieved in the previous discussion on the protein molecule I hope this can be continued

I would like to ask Dr Randle whether he has determined the insulin content of the plasma of normally growing children

P J RANDLE I have tested the plasma of one normally growing child as a control for one of my patients with pituitary gigantism The level was a little higher than normal but not significantly different from normal I might just mention in passing that I have also examined the plasma from a patient who developed mild diabetes in two successive pregnancies The diabetes subsided after the first pregnancy but was evident again after 32 weeks of the second pregnancy During the 36th week when the patient was not receiving insulin I estimated the insulin activity and it was at levels which I have found in acromegaly She subsequently gave birth to a baby which weighed 9 pounds 7 ounces Four weeks after delivery the diabetes had disappeared and insulin activity of the plasma was back to normal levels

R E HAIST I would like to point out however that if the insulin level is a regulating factor as far as islet function is concerned then under stimulation with growth hormone it is too much to expect an increase of insulin level in the blood It would not follow necessarily

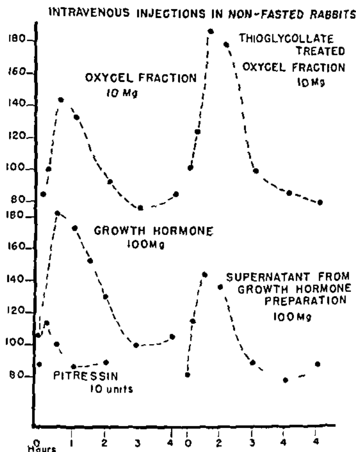


FIG 9

of Figure 8 under thioglycollate treated oxycell fraction one will note that we still obtained the fall in blood sugar while the rise was essentially gone In the section on the right (Fig 8) one will note that pitressin caused in ten minutes a very sharp rise in blood sugar as described some 30 years ago by Geiling I believe As little as 0 01 unit of pitressin will cause a significant 10 minute rise

In our studies with rabbits the patterns have been different as seen in Figure 9 Ten units of pitressin intravenously resulted in the same type of blood sugar pattern (lowest curve Fig 9) as observed in the rat i e a rapid 10 minutes rise and fall although not very large With 10 mg of the oxycel fraction of pituitary however, the blood sugar rise was maximal at one half hour and was still up at one hour When we treated that preparation with thioglycollate the substance which induced the rise in blood sugar was not destroyed and we observed the same type of response Growth hormone gave the same pattern and the supernatant from growth hormone gave the same pattern Accordingly the matter seems a bit confusing and

Part V

Influence of Growth Hormone on the Mammary Gland and on Human Metabolism

Chairman

Oscar Riddle

Formerly of the Carnegie Institution of Washington
Station for Experimental Evolution
Cold Spring Harbor New York

26

Mammary Growth and Lactation in Male Rats

W R Lyons R E Johnson R D Cole and C H Li
Department of Anatomy and the Hormone Research Laboratory University of California Berkeley

Since growth of the mammary gland and lactation are phenomena which are primarily if not entirely dependent upon hormones the choice of the sex of the experimental animal in which to study mammary stimulation by exogenous hormones would seem to be of little importance It is well known from experimental work and clinical observation that both sexes may exhibit complete mammary growth and lactation It would seem quite unlikely that in both sexes the cellular substrate upon which the hormones act is identical since there are undoubtedly slight fundamental differences between all female cells and their male counterparts Thus one might anticipate that the response of female mammary *anlagen* to hormones would differ slightly from that of the male tissue

The experiments exported herein were designed to show that one could use the recently accumulated knowledge about hormonal control of female mammary growth and function to reproduce in the male gland the various growth and secretory phases Some of the experiments therefore repeat and confirm what has already been shown in the glands of adult virgin rats namely that with pituitary and ovaries removed prolactational mammary growth may be stimulated readily by a combination of pituitary and ovarian hormones and that lactation may be induced in glands so prepared by a combination of pituitary and adrenocortical hormones For purposes of simplicity other important endocrine glands such as the pancreas liver parathyroid posterior pituitary and adrenal medulla have been excluded from consideration here

A report on the non-essentiality of the thyroid for mammary development and secretion will be given separately [1]

Briefly it has been shown that in hypophysectomized oophorectomized virgin rats mammary development simulating that of early pregnancy may

the latter preparation in hypophysectomized males as suggested by body weight increment following daily doses of 2 mg pointed to approximately 1% contamination with growth hormone but it was found that this activity was retained after the lactogenic hormone solution was boiled a procedure that promptly destroys purified preparations of growth hormone but not lactogenic hormone

Throughout the experiments the rats were given 3 0 ml of 5% glucose intraperitoneally every other day and on the two post-operative days they received also 5 mg of Terramycin® daily Estrone (E) and progesterone (P) were given separately and in combination in sesame oil in daily sub cutaneous doses of 1 μg and 4 mg respectively Hydrocortisone acetate (F) was administered subcutaneously in crystalline suspension The growth hormone was injected subcutaneously in saline and the lactogenic hormone was given subcutaneously usually in the form of an alum suspension at pH 5 0

The rats were weighed at 5 day intervals One day after the last injection necropsy was performed At this time mammary spreads were made from each animal and extramammary tissue was fixed and prepared for histologic study The spreads were stained *in toto* in alum carmine and embedded in plastic for future reference The development of the glands was judged (see Table 1) and graded on a 1 to 4 scale but due to variable responses in different sectors of the gland an arbitrary mean had to be used which involved the use of fractions of the 4 whole numbers The grade of alveolar distension with milk must necessarily be considered very arbitrary also It should be recalled that the male rate has no mammary nipples The operative sites were checked and if there was any doubt about the completeness of the operations the rats in question were excluded from the study

The first experiment was designed to test the efficacy of the hormonal combinations in doses comparable to those used in adult females In view of the fact that we were dealing with an almost rudimentary mammary gland in the 26 day-old operated male it was thought necessary to inject for a period of one month in order to imitate the growth processes of the normal female over the same age period Thus estrone progesterone lactogenic and growth hormones individually and in various combinations were injected for 30 days

During the course of the experiment it became apparent that the effects of the various mammary stimulating hormones could be ascertained quite readily after a two week period of injection The more effective combinations of hormones used in the first experiments were re run for 14 days (Table 1)

After it was learned that estrone at a dose level of 1μg plus growth hormone at a dose level of 0 2 mg were capable of inducing duct growth in the hypophysectomized gonadectomized males a series of three experiments using lower levels of growth hormone plus 1 μg of estrone were per

be induced by injecting estrone progesterone and lactogenic hormone (prolactin mammotropin)[2,3] Such animals show subnormal weight gain growth stasis or weight loss and if this is corrected by injecting growth hormone (somatotropin STH) as well as the other three hormones complete lobulo alveolar development equalling that of late pregnancy may be induced[3] Regardless of the degree of lobulo alveolar development mammary glands will lactate in these doubly operated rats after injections of lactogenic hormone growth hormone and either ACTH or cortisone When the adrenals are also removed cortisone instead of ACTH must be used with lactogenic hormone and growth hormone The only morphological prerequisite to the induction of milk secretion by this lactational triad is the secretory unit or alveolus Indeed after hypophysectomy and oophorectomy this triad will induce lactation in the few sparsely distributed alveoli that are present in the glands of the Long Evans virgin rat[4]

Our early experiments with the adult rat were concerned mainly with prolactational and lactational growth However in testing various combinations of the ovarian pituitary and adrenal hormones in the doubly (hypophysectomized gonadectomized) or triply (hypophysectomized gonadectomized adrenalectomized) operated rats it was noted that the preoperative virginal mammary *status quo* (ducts and a few small alveolar clusters) could be fairly well maintained with lactogenic hormone alone Growth hormone alone seems to have no effect in preventing regression but when it was given with estrone evidence of duct growth (but not alveolar development) was found in club like peripheral extensions

Experimental

These previous findings were useful in planning to reproduce in the male all of the main phases of mammary development Long Evans males hypophysectomized and gonadectomized shortly after weaning (age 26 days), were given hormone injections from the time of operation and for varying periods thereafter When the adrenals were also ablated this operation was performed three days after the other two It was a welcome finding that the triply operated rats fared well on 1% NaCl in their drinking water and on any of the hormonal regimens which included progesterone Only enough of the triply operated rats were studied to delineate the role of the adrenal in mammary growth and lactation The possible roles of the adrenal cortex in mammary growth were of less concern than was confirmation of earlier results in the female that the pituitary and ovarian hormones *require* no help from the adrenal in the developmental phases In some of the rats it was also necessary to control the possibility of mammary influencing steroids being formed in the adrenals under stimulus from the pituitary preparations

Growth hormone was prepared from ox pituitaries[5,6] and lactogenic hormone from sheep glands[7] both preparations had minimal detectable contaminations of other pituitary hormones A definite anabolic effect of

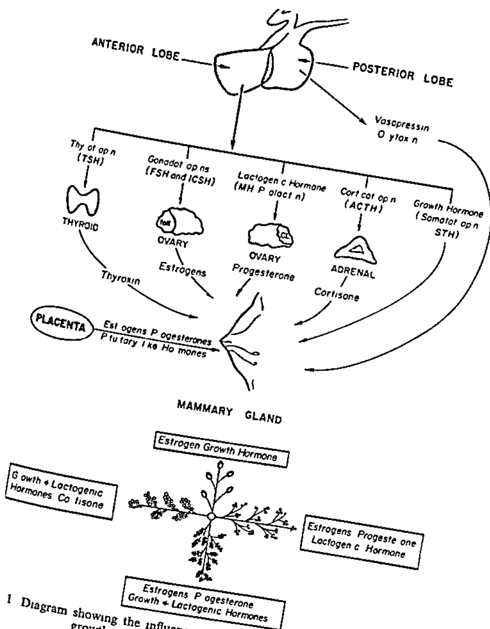


FIG 1 Diagram showing the influence of various agents on the development growth and function of the mammary gland

Table 1

THE EFFECT OF VARIOUS HORMONES INDIVIDUALLY AND IN COMBINATIONS ON THE MAMMARY GLAND OF HYPOPHYSECTOMIZED-GONADECTOMIZED MALE RATS*

Group	*Fig*	*No of Rats*	*Est µg*	*Prog mg*	*MH mg*	*STH mg*	*F mg*	*Days*	*State of Mammary Gland*
1	4	3	0	0	0	0	0	30	D—regressing
2	11	3	0	0	2	0	0	14	DAB 1+
		3	0	0	2	0	0	30	DAB 1+
3	6	3	0	0	0	0 2	0	30	D—regressing
4	5	2	1	0	0	0	0	14	D—
		3	1	0	0	0	0	30	D—
5		3	0	4	0	0	0	30	D—
6	9	3	1	4	0	0	0	30	D—
7		3	1	0	2	0	0	14	DAB 1+
	12	3	1	0	2	0	0	30	DAB 1+
8	13	3	0	4	2	0	0	14	DAB 1+
9	14	3	0	0	2	0 2	0	14	DAB 2+
10	7	3	1	0	0	0 02	0	14	D
	8	3	1	0	0	0 04	0	14	DAB 1+ and C
		3	1	0	0	0 08	0	14	DAB 1+ and C
		3	1	0	0	0 2	0	30	DAB 1+ and C
11	15	3	1	0	2	0 04	0	14	DAB 3+ and C
12		3	1	4	0	0 04	0	14	DAB 1+ and C
	10	3	1	4	0	0 2	0	30	DAB 1+ and C
	19	3†	1	4	0	0 2	0	21	DAB 2+ and C
13		2	1	4	1	0	0	14	DAB 2+ and LA 0 5+
	16	9	1	4	2	0	0	14	LA 0 5+ to 2+
		3	1	4	2	0	0	30	LA 0 5+ to 2+
	20	4†	1	4	2	0	0	21	LA 1+ to 2+
14		2	1	4	1	0 04	0	14	LA 1 5+ and 2 5+
		3	1	4	2	0 04	0	14	LA 1 5+ to 2 5+
	17	3	1	4	4	0 04	0	14	LA 3 5+
		3	1	4	2	0 2	0	30	LA 0 5+ to 2+
		2†	1	4	4	0 2	0	21	LA 2 5+ and 3+
15	18	3	1	4	4	0 04	0	14	
		then	0	0	4	0 04	1	3	LA 3+ and L 2+
		4	1	4	2	0 04	0	20	
		then	0	0	2	0 04	1	6	LA 3+ and L 4+
		3	1	4	2	0 2	0	30	
		then	0	0	5	0 5	1	7	LA 3+ and L 4+
	21	2†	1	4	2	0 2	0	21	
		then	0	0	5	0 5	1	5	LA 3+ and L 3+

* Est = estrone Prog = progesterone MH = mammotropin (lactogenic hormone) STH = somatotropin (growth hormone) F = hydrocortisone acetate d = ducts only C = pronounced club endings on ducts DAB = ducts and alveolar buds LA = degrees of lobulo alveolar development L = lactating (degrees of distention with milk)

† Also adrenalectomized

formed in an attempt to ascertain the approximate minimal effective dose of growth hormone (Table 1)

It was thought unnecessary to repeat this series in triply-operated rats (hypophysectomized gonadectomized and adrenalectomized) Hence with the latter only four small groups of males were studied using essentially the same technique as with the doubly operated animals These rats were hypophysectomized at 26 days of age and three days later their adrenals and testes were removed All of the adrenalectomized animals were given 1% NaCl for drinking purposes and every day they received 3 0 ml of 5% glucose in 0 85% NaCl intraperitoneally For the first five days during the operative stages 5 mg of Terramycin® were added to the daily dose of glucose saline All the rats reported in this experiment received 1 μg of estrone 4 mg of progesterone and either 2 mg of lactogenic hormone or 0 25 mg of growth hormone or both of the latter two hormones daily for 21 days They fared well on this regimen as did the two that were injected during the lactational phase of five days with 5 mg of lactogenic hormone 0 5 mg growth hormone and 1 mg of hydrocortisone

Results The results of the various experiments are recorded in Table 1 and are shown to better advantage in the photographs of Plates 1 and 2 Hypophysectomized gonadectomized rats are referred to as doubly operated and the hypophysectomized gonadectomized adrenalectomized as triply-operated

Group 1 are doubly-operated rats which received the usual supportive treatment but no hormones Sesame oil the vehicle for estrone and progesterone was injected subcutaneously The result obtained may be appreciated by comparing the developing gland of a normal 26 day-old rat (Fig 2) with the atrophic gland of a doubly operated rat injected with sesame oil (Fig 4) It will be noted that the larger ducts have persisted although the smaller branches appear to be wilting

Groups 3 4 5 6 and 10 (Section 1) may be combined for the purpose of reporting results because the glands of all of these doubly operated rats variously treated showed regression from a normal 26 day status Group 3

Fig 5 Same injected daily with 1 μg estrone (E) for 14 days regression

Fig 6 Same injected daily with 200 μg growth hormone (STH) for 30 days regression

Fig 7 Same injected daily with 20 μg STH plus 1 μg E for 14 days regression

Fig 8 Same injected daily with 40 μg STH plus 1 μg E for 14 days duct growth with club formation

Fig 9 Same injected daily with 1 μg E plus 4 mg progesterone (P) for 30 days regression

Fig 10 Same injected daily with 1 μg E plus 4 mg P plus 200 μg STH for 30 days duct growth with club formation similar to rats treated with E plus STH

Fig 11 Same injected daily with 2 mg MH for 14 days Maintenance

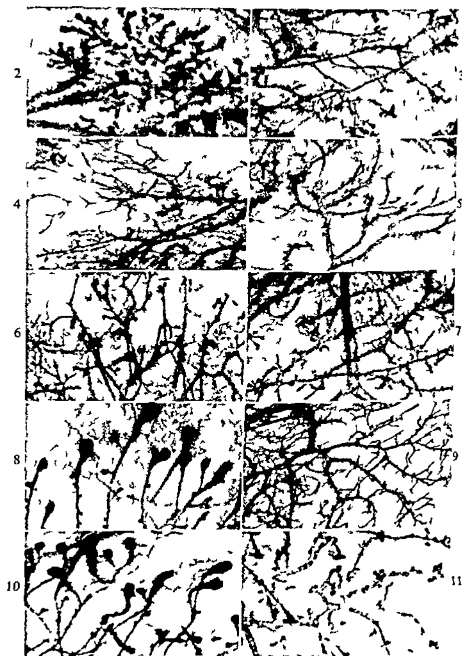


PLATE 1

Areas of the first right inguinal mammary glands (x 14) from Long Evans male rats

FIG 2 Normal 26 days old about ½ of the gland is shown
FIG 3 Normal 59 days old about ⅕ of the gland is shown
FIG 4 Hypophysectomized and gonadectomized at 26 days of age and injected with sesame oil for 30 days

(Fig 6) received only growth hormone group 4 (Fig 5) only estrone group 5 only progesterone, group 6 estrone and progesterone (Fig 9) and the first section of group 10 estrone and a subthreshold dose of growth hormone (Fig 7)

Groups 2 7 8 and 9 all composed of doubly operated rats may be reported together for here again the results were essentially similar in all four groups The glands showed maintenance of the ducts and alveolar buds apparently due to lactogenic hormone alone (group 2 Fig 11) There was a suggestion of better maintenance with lactogenic hormone plus growth hormone (group 9 Fig 14) But neither estrone (group 7 Fig 12) nor progesterone given with lactogenic hormone (group 8 Fig 13) promoted better maintenance than lactogenic hormone alone

Groups 10 (Sections 2–4) 11 and 12 showed essentially similar growth responses In all the synergism between estrone and growth hormone was clearly demonstrated in the remarkable end bulb proliferation The results with three levels of growth hormone (40–200 µg) plus 1 µg of estrone (group 10 Fig 8) or 1 µg of estrone and 4 mg of progesterone (group 12 Fig 10) were the same It was tempting to interpret some of these results as representing only duct growth but there were present small lateral buds that seem to be able to develop as ducts with the proper stimulus or to secrete when influenced by the lactational trio (lactogenic hormone growth hormone and ACTH or cortisone) The designation DAB or ducts with alveolar or lateral buds was therefore used It seemed significant that the triply-operated rats with no adrenal hormone except what is sometimes consider d to be corticoid (progesterone) showed good growth of end bulbs in response to the combination of estrone and growth hormone in

---

Fig 14 Same injected daily with 2 mg MH plus 200 µg STH for 14 days maintenance

Fig 15 Same injected with 1 µg E plus 2 mg MH plus 40 µg STH for 14 days duct growth with club formation similar to E plus STH

Fig 16 Same injected daily with 1 µg E plus 4 mg P plus 2 mg MH for 14 days 1 + lobulo alveolar (LA) growth

Fig 17 Same injected daily with 1 µg E plus 4 mg P plus 4 mg MH plus 40 µg STH for 14 days 3 + LA growth

Fig 18 Same injected daily for 14 days with 1 µg E plus 4 mg P plus 4 mg MH plus 40 µg STH then 4 mg MH plus 40 µg STH plus 1 mg hydrocortisone acetate (F) for 3 days 4+ LA growth and lactation

Fig 19 Hypophysectomized gonadectomized and adrenalectomized at 26 days of age and injected daily with 1 µg E plus 4 mg P plus 200 µg STH for 21 days duct growth with club formation

Fig 20 Same injected daily with 1 µg E plus 4 mg P plus 2 mg MH for 21 days 2+ LA growth

Fig 21 Same injected daily for 21 days with 1 µg E plus 4 mg P plus 2 mg MH plus 200 µg STH and then for 5 days with 5 mg MH plus 500 µg STH plus 1 mg F LA 3 5+ and lactation

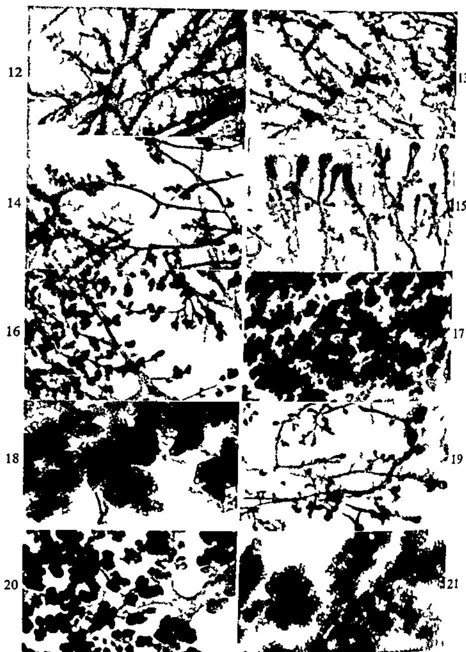


PLATE 2

Areas of the first right inguinal mammary gland (x 14) from Long Evans male rats

FIG 12 Hypophysectomized and gonadectomized at 26 days of age and injected daily with 1 μg E plus 2 mg lactogenic hormone (MH) for 30 days maintenance

FIG 13 Same injected daily with 4 mg P plus 2 mg MH for 14 days maintenance

ovarian and pituitary like hormones would complicate this report However the picture of normal breast development in pregnancy cannot be understood precisely unless the placental factors be included Evidence has been adduced[9] that the midterm rat placenta imitates the pituitary rather closely in its mammary influences

The mechanism whereby the five anterior pituitary hormones (lactogenic hormone ACTH growth hormone FSH and ICSH) influence mammary development and subsequent lactation may thus be outlined (Fig 1) progesterone is formed by the corpus luteum under the influence of lactogenic hormone estrogen is secreted from the ovary through the stimulation of FSH plus ICSH combined in optimal proportions ACTH will stimulate the cortex to produce a variety of steroids three of which (DOCA F plus F) as we know from this and other work will enhance the effects of lactogenic hormone plus growth hormone on the milk secretory process The mammary stimulating action of growth hormone has been revealed in these experiments however until work has been done on mammary tissue culture or on isolated organ preparations the nature of this hormone s activity in this regard will remain conjectural

Furthermore as summarized in Figure 1 the following changes in the mammary gland are induced by estrone progesterone lactogenic hormone growth hormone and hydrocortisone acetate (F) in immature male rats with pituitaries and testes removed

1 *Regression* after injection of (a) Sesame oil (b) 200 μg of growth hormone (c) 1 μg of estrone (d) 4 mg of progesterone 1 μg of estrone plus 4 mg of progesterone, (e) 1 μg of estrone plus 4 mg of progesterone plus 20 μg of growth hormone

2 *Maintenance* after (a) 2 mg of lactogenic hormone, (b) 1 μg of estrone plus 2 mg of lactogenic hormone (c) 4 mg of progesterone plus 2 mg of lactogenic hormone (d) 2 mg of lactogenic hormone plus 200 μg of growth hormone

3 *Duct proliferation* in the form of large end bulbs after (a) 1 μg of estrone plus 40 80 or 200 μg of growth hormone (b) 1 μg of estrone plus 2 mg of lactogenic hormone plus 40 μg of growth hormone (c) 1 μg of estrone plus 4 mg of progesterone plus 40 or 200 μg of growth hormone The (c) regimen induced the same changes in rats which had also been adrenalectomized

4 *Limited lobulo-alveolar growth* after 1 μg of estrone plus 4 mg of progesterone and 1 or 2 mg of lactogenic hormone The same results were obtained when the adrenals also had been removed

5 *Full lobulo-alveolar growth* after 1 μg of estrone plus 4 mg of progesterone plus 4 mg of lactogenic hormone plus 40 μg of growth hormone In rats with adrenals also ablated 1 μg of estrone plus 4 mg of progesterone plus 4 mg of lactogenic hormone plus 200 μg of growth hormone induced good lobulo-alveolar growth

jected together with the progesterone (group 12, section 3 Fig 19) The rats which received estrone plus lactogenic hormone and growth hormone showed good club formations at the ends of the main ducts and a greater degree of lateral budding (group 12 Fig 15)

Group 13 was made up of doubly- or triply operated rats injected with estrone progesterone and lactogenic hormone This together with previous experience informed us that 1–2 mg was an adequate daily dose of lacto genic hormone for synergism with estrone and progesterone leading to in complete but definite lobular alveolar development (group 13 Fig 16) The adrenalectomized rats responded equally as well as the doubly operated animals (Fig 20)

Group 14 was composed of 11 doubly operated and 2 triply operated rats that received estrone progesterone and different levels of lactogenic hor mone plus growth hormone When the level of estrone was held at 1 µg that of progesterone at 4 mg and that of growth hormone at 40 µg, and when that of lactogenic hormone was varied from 1 to 4 mg daily the higher dose of the latter was shown to induce greater lobulo alveolar growth (Fig 17) This degree of development is equivalent to that attained in late pregnancy in the rat Again the adrenal was unnecessary although here also progesterone must receive its due consideration as a corticoid

Group 15 consisted of 13 doubly operated and 2 triply operated rats given the prolactational quartet of hormones estrone progesterone growth hormone and lactogenic hormone for periods of 14–30 days and then given the lactational group growth hormone lactogenic hormone and hydro cortisone As shown in the Table and Figures 18 and 21 the response was one of full secretory activity in well developed glands

Discussion

In these experiments some of which may be said to have *confirmed* earlier findings in the adult female rat and other animal forms there has been an attempt to emphasize the importance of multiple synergisms in mammary growth and milk secretion It must be admitted that this is more easily accomplished than the task of defining the precise and specific action of each individual pure hormone

To schematize the play of pituitary and target organ hormones *upon the* mammary gland a chart has been prepared (Fig 1) It would seem that six anterior pituitary hormones as well as the posterior lobe twins have some direct or indirect influence upon mammary growth and lactation In the work presented herein nothing has been done to demonstrate posterior lobe or thyroid activity Such functions have been treated elsewhere [8] The work of Chen et al [1] proves the non necessity of TSH or thyroid substances for mammary growth and secretion but this may not be said to deny a certain usefulness of these substances in normal function

To present the evidence for the placental storage or manufacture of

27

Effects of Somatotropin and Other Pituitary Hormones on the Lactating Mammary Gland

S J Folley

National Institute for Research in Dairying University of Reading England

Galactopoietic Action of Somatotropin

It has been well known for over 25 years that anterior pituitary extracts contain factors capable of increasing the milk yield of cows in declining lactation This phenomenon an example of which is shown in Figure 1 illustrates the so-called galactopoietic action of pituitary extracts (for terminology see refs 1 and 2) It has long been a matter of considerable interest to find out which of the well-characterized anterior pituitary hormones are responsible for the galactopoietic properties of unfractionated pituitary extracts A solution of this problem has been attempted in joint studies by F G Young and myself with various collaborators over a number of years The earlier work is discussed in reviews by Young[3] and Folley and Young [4]

Soon after the discovery by Stricker and Grueter[5] of the existence of the anterior pituitary lactogenic hormone which I shall here call prolactin and its characterization by Riddle Bates and Dykshorn [6] it was believed that prolactin must possess what we now call galactopoietic properties However the work of Azimov and Krouze[7] and more particularly the early work of Folley and Young[8] suggested that ox anterior pituitary extracts must contain some component or components distinct from prolactin with more powerful galactopoietic properties perhaps capable of acting alone or perhaps synergistically with other anterior pituitary factors such as prolactin itself the combination thus forming a potent galactopoietic complex These findings gave rise to the belief that the galactopoietic potency of purified prolactin was not very great at least in the cow As will be seen later this belief has been supported by subsequent work

6 *Milk secretion* after as few as 14 days of *preliminary treatment* with 1 μg of estrone plus 4 mg of progestrone plus 4 mg of lactogenic hormone plus 40 μg of growth hormone followed by as few as 3 days of injections with 4 mg of lactogenic hormone plus 40 μg of growth hormone plus 1 mg of F Rats that had also been adrenalectomized showed excellent milk secretion under a similar regimen

To be complete the schema should show a regressing or atrophic gland The picture would consist only of branching lines representing shrinking ducts bare of buds

Summary

In hypophysectomized gonadectomized (and in some cases adrenalectomized) immature male rats mammary duct growth was induced by injecting estrone and growth hormone

Limited lobulo alveolar mammary growth qualitatively comparable to that of early pregnancy was induced by injecting estrone progesterone and lactogenic hormone

Full lobulo alveolar development qualitatively comparable to that of late pregnancy was induced by injecting estrone progesterone lactogenic hormone and growth hormone

In glands in which lobulo alveolar (or prolactational) growth had been stimulated by the just mentioned quartet of hormones milk secretion was induced by a lactational trio of lactogenic hormone growth hormone and hydrocortisone acetate

Most of the life history of the female mammary gland has thus been reproduced on a slightly reduced scale in male rats

References

1 Chen T Johnson R E Li C H Cole R D and W R Lyons In manuscript
2 Lyons W R *Essays in Biology* Berkeley Univ of Calif Press 1943 315
3 Lyons W R Li C H and R E Johnson *J Clin Endocrinol and Metabolism* 12 45 (1952)
4 Lyons W R Li C H Johnson R E and R D Cole Unpublished
5 Li C H Evans H M and M E Simpson *J Biol Chem* 159 353 (1945)
6 Li C H *J Biol Chem* (in press)
7 Lyons W R *Cold Spring Harbor Symposia Quant Biol* 5 198 (1937)
8 Folley S J *Marshall s Physiology of Reproduction* Ed A S Parkes New York Longmans Green & Co 1952 525
9 Ray R D Averill S C Lyons W R and R E Johnson *Proc Soc Exp Biol Med* (in press)
10 Lyons W R Li C H Cole R D and R E Johnson *J Clin Endocrinol and Metabolism* 13 836 (1953)

milk yield The inhibitory effect of ACTH on lactation in the cow has been confirmed in recent experiments in our laboratory (Flux Folley and Rowland)[11] in which purified ACTH of low molecular weight was used and also by Shaw Chung and Bunding[1] The activity of the purified somatotropin in these single injection tests was quantitatively sufficient to account for all or nearly all of the activity displayed by our unfractionated extracts of ox anterior pituitary when considered in relation to their estimated content of somatotropin In tests of longer duration involving repeated injections it is probable that stimulation of the thyroid by thryrotropin might contribute to the total galactopoietic effect since thyroid hormone is a well known galactopoietic agent in the cow This would not necessarily apply only to unfractionated anterior lobe extracts which we can now recognize we had used in our earlier work essentially as a crude somatotropin preparation but might also apply to work with purified somatotropin since it is notoriously difficult to free the latter from thyrotropin

The galactopoietic activity of growth hormone is probably of fundamental physiological significance Young[3] pointed out that growth milk secretion and diabetogenesis are all processes which involve a restraint on

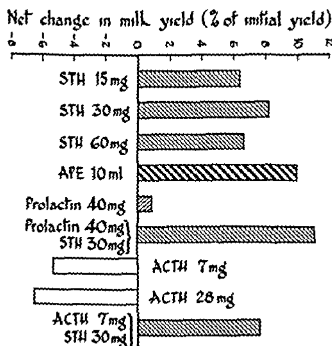


FIG 2 Effect of single injection of various ox anterior pituitary preparations on the milk yields of groups of cows on three farms The bars show the change in yield observed during the two days following the injection expressed as a percentage of the initial yield corrected for the change in yield for control cows (From Cotes et al [10])

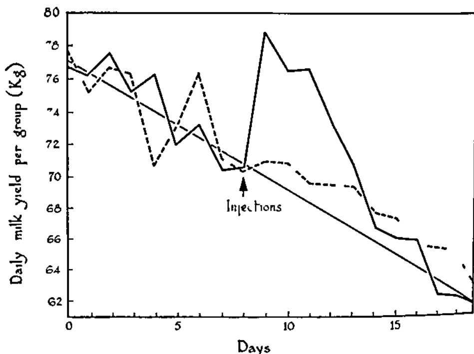


FIG 1 Galactopoietic effect of a single injection of 10 ml unfractionated ox anterior pituitary extract on the milk yield (unbroken line) of a group of four cows The dotted line shows the milk yield of a group of four control cows injected with physiological saline (From Folley and Young[5])

In the course of these studies during which a good number of anterior pituitary extracts mainly from the ox were studied it was noticed that although in agreement with what has just been said the relation between galactopoietic potency and prolactin content as measured by the pigeon crop assay was not very close there seemed to be a close relationship or at least association between diabetogenic activity and galactopoietic potency On reflection this was not altogether surprising because a number of analogies could be drawn from the biochemical standpoint between diabetes and lactation (see 3) Therefore when purified somatotropin was found by Cotes Reid and Young[9] to exhibit diabetogenic properties in the intact cat it seemed likely that somatotropin would prove to be galactopoietic Accordingly Young and I decided to examine purified somatotropin for galactopoietic properties in lactating cows In experiments on groups of lactating cows on three farms Cotes Chrichton Folley and Young[10] found that single injections of purified somatotropin caused appreciable and statistically significant temporary increases in the milk yield (Fig 2) On the other hand single injections of approximately 1 000 I U (40 mg) purified prolactin had no detectable galactopoietic effect while single injections of ACTH protein in the doses used actually caused temporary decreases in

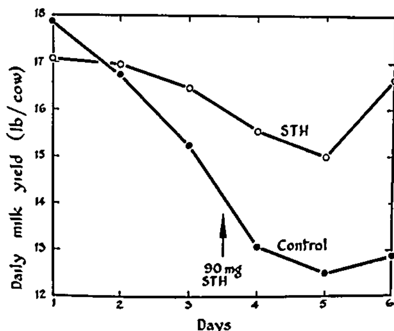


FIG 3 Milk yield curves for a group of 5 cows receiving a single injection of 90 mg somatotropin (Armour R 377174) and for a group of 5 control cows injected with saline

Two single injection experiments with the same somatotropin preparation were also carried out by Dr Flux on lactating goats of the British Saanen breed randomized block designs being again used The results of the first experiment are summarized in Table 2 Three doses of somatotropin (10

Table 2

EFFECT OF A SINGLE INJECTION OF STH (ARMOUR R377174) ON THE MILK YIELD OF GOATS
(6 goats on each treatment)

Dose of STH (Armour Units)	*Mean Milk Yield (lb /goat/day)*		*Response (lb /goat/ day)*	*Per Cent Response*
	2 Days before Injection	*2 Days after Injection*		
0	6 42	6 19		
10	5 66	6 83	0 64	10 9
20	5 93	6 19	0 00	0 0
30	5 53	6 31	0 12	2 0

Responses significant at 10% level
Coefficient of variation 7 0%

* Adjusted on basis of covariance analysis

the oxidation of metabolites which are thus made available for other purposes such as milk formation so that a close relationship between the hormonal mechanisms involved in the control of all three phenomena is to be expected

The galactopoietic effect of somatotropin has been confirmed in cows by Donker and Petersen[13 14] in single injection tests and also by Chung Shaw and Gill [15] Shaw Chung and Bunding [1] Wrenn and Sykes[16] and Brumby and Hancock[17] who have observed substantial increases in milk yield in response to series of daily injections Confirmatory results have also been obtained in lactating ewes by Jordan and Shaffhausen [18]

Table 1

EFFECT OF A SINGLE INJECTION OF STH (ARMOUR R377174) ON THE MILK YIELD OF COWS IN ADVANCED LACTATION
(5 cows on each treatment)

	Mean Milk Yield (lb /cow/day)		*Change in Yield (lb /day)*	*Per Cent Response (Treated Control)*
	3 Days before Injection	*3 Days after Injection*		
Control	16 6	12 8	−3 8	
STH 90 Armour units	16 8	15 7	−1 1	16 1

Response not significant $P \simeq 0\ 2$

By contrast in experiments recently carried out in our laboratory by Dr D S Flux rather puzzling results have been obtained We thought it would be of interest to see if somatotropin would temporarily increase the milk yield of cows in rather late lactation This possibility was investigated in an experiment on two groups of five cows in which Dr Flux found that single injections of 90 mg (90 Armour units) of purified somatotropin (Armour R377174) had no significant effect on the milk yield A randomized block design was used for this experiment the results of which are summarized in Table 1 At first sight the results in the Table might appear to indicate that there was an appreciable galactopoietic response to the injections This arises from the fact that while as shown in Figure 3 the milk yields of both control and hormone injected groups declined over the observation period the former declined faster than the latter It may be noted however that the milk yield curve for the group receiving somatotropin considered by itself provides no evidence that the treatment had any effect whatever Covariance analysis showed that the calculated response given in the last column of Table 1 was not significant and also revealed that the accuracy of the experiment was not very great probably because the cows were approaching the end of lactation It seems safest to conclude that there was no response to the treatment

although it must be noted that Shaw et al [1] obtained positive responses in cows in advanced lactation which on the average were giving only 15 lb milk per day As regards the goats it may be significant in the present connection that in experiments carried out at about the same time as those just described (Flux unpublished) other lactating goats from the same pedigree herd which had been spayed in infancy and then brought into lactation by hormone treatment proved almost completely refractory to single injections of 1 mg L thyroxine or of 5 ml unfractionated ox anterior pituitary extract (1 ml ≃ 250 mg fresh tissue) both of which agents are regularly effective in cows Incidentally they were also refractory to single injections of 1 000 I U purified prolactin but this is not significant in the present connection since the galactopoietic activity of prolactin seems to be relatively small as judged by the poor responses in cows obtained by a number of workers (Cotes et al [10] Donker and Petersen [14] Biavati and Fiori [19] Wrenn and Sykes[16]) It may be that somatotropin is not a limiting factor in declining lactation in the goat though this apparently does not apply to a close relative the sheep in which animal Jordan and Shaff hausen[17] obtained positive galactopoietic responses with somatotropin Another possibility is that simultaneous administration of insulin would have permitted a galactopoietic response to somatotropin There is now considerable experimental support (see 20) for the view put forward some years ago by Young that the growth response to somatotropin requires a sufficiency of insulin in the absence of which the pituitary growth hormone exerts a diabetogenic action If the same applies to the galactopoietic response it seems possible that in some circumstances galactopoiesis might fail because of a deficiency of insulin in which case insulin would be expected to permit or enhance the response to somatotropin In any event for the above reasons the experiments just discussed do not appear to provide any grounds for questioning the reality of the galactopoietic action of purified somatotropin in the cow particularly since the original work of Cotes et al [10] has been so amply confirmed by other workers The possibility that the galactopoietic activity of somatotropin preparations is due not to the hormone itself but to some hitherto undetected contaminant remains for further consideration

Effects of Somatotropin on Mammary Tissue *in Vitro*

Mammary tissue is outstanding for its ability to synthesize fatty acids from small molecules even *in vitro* as shown by the fact that if mammary gland slices from lactating rats are incubated with 1 C^{13} 2 tritio acetate plus C^{14} glucose fatty acids rich in all three isotopes can be isolated from the slices (Balmain Folley and Glascock [1]) That this phenomenon signifies net fatty acid synthesis rather than merely a rapid turnover is evident from the fact that rat mammary gland slices provided they come from lactating animals utilize glucose or glucose plus acetate *in vitro* with R Q > 1 (Folley

20 and 30 mg) were tried and there were six goats on each treatment The responses given in the last column were estimated from the adjusted post injection yields which were calculated by covariance analysis The accuracy of this experiment was satisfactory the coefficient of variation being no more than 7 0% The results are puzzling in that while the lowest of the three doses tried (10 mg) appeared to give a galactopoietic response though this only achieved a 10% level of significance the two higher doses were without effect It was decided to investigate the lower range of doses further Accordingly a second experiment was carried out a week later on the same animals in which the responses to single injections of 5 and 10 mg respectively of the same preparation were studied In this case there were seven goats on each treatment The results are given in Table 3 This time a single injection of 10 mg of the somatotropin preparation had no appreciable galactopoietic effect though 5 mg gave a small but not statistically significant response

Table 3

EFFECT OF A SINGLE INJECTION OF STH (ARMOUR R377174) ON THE MILK YIELD OF GOATS
(7 goats on each treatment)

Dose of STH (Armour Units)	*Mean Milk Yield (lb /goat/day)*		*Change in Yield (lb /day)*	*Per Cent Response*
	2 Days before Injection	*2 Days after Injection**		
0	5 88	5 67		
5	5 97	6 23	0 56	9 7
10	5 51	5 75	0 08	1 4

Responses significant at 10–20% level
Coefficient of variation 8 8%

* Adjusted on basis of covariance analysis

There seem to be two possible explanations of these negative or virtually negative findings in the cow and the goat Either the somatotropin preparation was inactive or the animals used were refractory to the galactopoietic action of somatotropin The first explanation seems unlikely unless galactopoietic properties are not characteristic of somatotropin itself but are due to a contaminant since in semi quantitative tests this somatotropin preparation caused prompt resumption of growth in hypophysectomized rats Moreover the manufacturers have since informed me that material from the same batch has given positive galactopoietic responses in cows in experiments carried out elsewhere It seems more likely that the experimental animals were refractory to the galactopoietic actions of somatotropin In the case of the cows this might be because they were in late lactation

Table 4

EFFECT OF SOMATOTROPIN (ARMOUR NO M 308) ON THE RESPIRATION OF MAMMARY GLAND SLICES FROM LACTATING RATS

	No of Repls	R Q	$-Q_{O_2}$	Q_{CO_2}	Q	Q_i	-Q
				(a) substrate glucose			
Control	20	1 54	11 7	18 1	+2 5	1 92	9 2
STH	20	1 67	12 6	21 1	+2 2	1 74	10 8
P*		<0 001	0 2>P>0 05	<0 001	0 2>P>0 05	0 01>P>0 001	0 01>P>0 001
				(b) substrate glucose + acetate			
Control	20	1 51	10 4	15 9	−1 4	0 37	6 9
STH	20	1 59	11 3	18 3	−1 7	0 44	7 8
P*		0 01>P>0 001	0 2>P>0 5	0 05>P>0 01	>0 2	>0 2	>0 2

* Probability levels for the observed differences tested separately

and French [3]) Addition of insulin (glucagon free) to the medium causes the R Q of the slices to increase from a value of 1 40–1 57 to a value of 1 89–2 15 it also increases $-Q_{O_2}$ $-Q_{acetate}$ and $-Q_{glucose}$ (Balmain and Folley[24]) This, taken together with the fact that the rate of incorporation of acetate and glucose carbon into the mammary gland fatty acids is increased by insulin (Balmain Folley and Glascock [1 5]) justifies the conclusion that insulin increases the rate of lipogenesis from acetate and glucose in mammary tissue of the rat In consequence of the high R Q exhibited by lactating mammary gland slices when incubated in Warburg vessels with suitable substrates dissolved in saline bicarbonate in equilibrium with O_2/CO_2 mixture, the gas exchange is such that there is a net gas output (calculated as CO_2) and the curve of total gas pressure against time (composite respiration curve) rises steadily from the outset of the incubation period (Balmain and Folley [6]) As might be expected from its effect on the R Q addition of insulin increases the slope of the composite respiration curve (Balmain French and Folley [7] Balmain Folley and Glascock [5]) a response which can be used for insulin assay *in vitro* (Balmain Cox Folley and Mc Naught [9])

In view of the diabetogenic action of somatotropin it might be expected that the latter would by itself evoke effects on the metabolism of rat mammary tissue in the opposite sense to insulin and also might antagonize the action of insulin when both hormones are added to the incubation medium On the other hand growth hormone has been reported to display insulin like effects under some circumstances both *in vivo* (e g Milman and Russell[30]) and on the rat diaphragm equilibrated with it *in vitro* (Ottaway [31] Bulbrook and Ottaway[3]) In unpublished experiments carried out in our laboratory Dr Judith Balmain was unable to demonstrate any significant effect of a purified somatotropin preparation (500 μg /ml) on the metabolism of rat mammary gland slices as judged by the slope of the composite respiration curve nor did this preparation modify in any way the marked action of insulin thereon However more recently in our laboratory Dr Mary McNaught has found that somatotropin (Armour no M308) *in vitro* at a concentration of 100 μg /ml will significantly increase the R Q of lactating rat mammary gland slices incubated with glucose as substrate This increase in R Q was accompanied by increases in Q_{CO_2} $-Q_{O_2}$ and $-Q_{gl}$ while the glycolysis (Q_{lactat}) was reduced (Table 4) These effects of somatotropin were somewhat attenuated if acetate was present as well as glucose we are at present unable to explain why acetate decreased the stimulating effect of somatotropin on the R Q This complex of effects of somatotropin on the metabolism of the lactating rat mammary gland slice is in all respects similar to that evoked by insulin[24] and would seem to be opposite in a sense to the effects of somatotropin on the R Q of the isolated diaphragm of the fasted rat described by Recant [33] As regards glucose utilization we appear to be dealing with an effect essentially similar to that

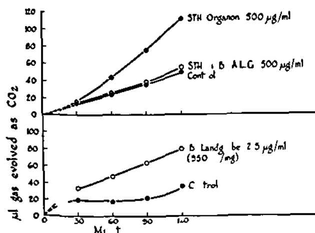
Table 5

EFFECTS OF PITUITARY HORMONES ON THE COMPOSITE RESPIRATION CURVE OF RAT MAMMARY GLAND SLICES

Type of Preparation	*Total No Tested*	*No Positive*	*No Negative*
Prolactin	10	9	1
STH	16	7	9
ACTH	9	5	4
TSH	2	2	0
FSH	1	0	1
B (intermedin)	6	6	0
Oxytocin	2	0	2
Vasopressin	1	1	0
PLP protein (Van Dyke)	1	1	0

At maximum dose level of 500 µg ml positive preparations gave significant ($P<0\ 05$) increase in net gas evolution

medin preparations was undertaken therefore in collaboration with Professor F W Landgrebe and Dr G M Mitchell As is shown in Table 5 of six intermedin preparations of varying degrees of purity which were tested all were positive when tested at concentrations of 500 µg ml or below Experiments to determine the minimum concentration necessary to give a detectable response under our conditions provided the most cogent reason for our belief that the activity was due to intermedin itself since the



Students *t* test on results at 120 min control vs STH + B 0 5 > P > 0 4
STH Organon 0·05 > P > 0 02
B 0 05 > P > 0 02

FIG 4 Effects of somatotropin and intermedin *in vitro* on the composite respiration curve of mammary gland slices from lactating rats (substrates acetate + glucose)

reported by Bulbrook and Ottaway[38] for the isolated rat diaphragm in the presence of the same concentration of somatotropin According to these investigators, the nature of the effect of somatotropin *in vitro* on the glucose uptake of the isolated diaphragm of the rat is a function of the concentration of somatotropin when present in concentrations of 75 μg /ml and upwards somatotropin increased the glucose uptake of the diaphragm while in lower concentrations the hormone had the opposite effect The effect of somatotropin in relatively low concentrations on the R Q and glucose uptake of mammary slices is under investigation in our laboratory

Doubts have been expressed from time to time whether the various biological activities exhibited by purified preparations of somatotropin are due to one and the same chemical entity The implication is that in some cases an allegedly highly purified preparation of crystalline growth hormone may not be a pure homogeneous substance but, rather may consist of a mixture of distinct molecular species possessing different biological activities This possibility has to be considered in respect of the above mentioned effects of somatotropin on the R Q and glucose uptake of rat mammary gland slices for the following reason We have found that equilibration with prolactin increases the (positive) net gas output of mammary gland slices taken from rats killed in early lactation thus increasing the slope of the composite respiration curve (Balmain and Folley [8]) Though these results have been confirmed by Mr T R Bradley in further extensive studies in our laboratory on a number of prolactin preparations (see Bradley Folley Landgrebe and Mitchell[34]) certain reasons gradually emerged why it seemed unlikely that the effect could be due to prolactin itself Thus the minimum effective concentration of prolactin was rather high (*ca* 200 μg /ml) and further there was no obvious relation between prolactin potency (crop test) and the effect on the net gas output of the mammary gland slice This led to the examination of a number of other pituitary preparations of various types the outcome being that the effect was found to be given by many preparations of various well recognized anterior pituitary hormones (see Table 5)

Of particular interest to the present discussion is the fact that as will be seen in the Table seven out of a total of sixteen purified somatotropin preparations proved to be positive when tested for their effects on the slope of the composite respiration curve of rat mammary gland slices at concentrations of up to 500 μg /ml A similar state of affairs was found to apply to ACTH preparations some but not all of those tested being found active In short the results summarized in Table 5 taken together suggest that the response under discussion is due to a substance present as an impurity in those anterior lobe preparations which gave positive responses In view of this fractions from other lobes of the pituitary were tested (Table 5) and it soon became apparent that preparations of intermedin (B) were especially active More detailed examination of inter

tissue just as the two effects are related in the case of insulin These findings pose the question whether the action of our somatotropin preparation in raising the R Q of lactating rat mammary tissue is an intrinsic property of somatotropin itself or whether it is due to intermedin present as an impurity particularly since the preparation used in our R Q studies is quite active in increasing the slope of the composite respiration curve This ques tion is still under study in our laboratory but it can be said that at the time of writing we are inclined to the first alternative Our reason for this belief is that the mechanism of the increase in the R Q (substrates acetate + glucose) evoked by somatotropin and intermedin respectively does not appear to be the same Somatotropin increased both $-Q_O$ and Q_{CO} but did not increase the glycolysis the increase in R Q evoked by inter medin on the other hand was the result of a decrease in $-Q_{O_2}$ accompanied by a slight increase in Q_{CO_2} The acid uptake was also markedly increased These findings are not conclusive since the points of difference are small and the respective measurements of the effects of the two hormones were made on tissue from rats killed at somewhat different stages of lactation Further studies will be necessary to clear up this question

Acknowledgments

The bulk of the somatotropin used in the unpublished experiments de scribed in this paper was kindly provided by Dr Irby Bunding of the Armour Laboratories and of the intermedin by Professor F W Landgrebe The paper refers briefly to tests carried out on various pituitary preparations which were generously furnished by numerous investigators and by repre sentatives of pharmaceutical manufacturers To all the persons concerned too numerous to mention by name here I express my sincere thanks I hope it will be possible to acknowledge them by name elsewhere Dr D S Flux participated in the work while holding a National Research Fellowship of the Department of Scientific and Industrial Research New Zealand and Mr T R Bradley while holding a Research Fellowship from Organon Laboratories Ltd Valuable advice and help in some statistical matters has been given by Mr C P Cox to whom my thanks are due

References

1 Bergman A J and C W Turner *J Dairy Sci* **23** 1229 (1940)
2 Folley S J and F G Young *J Endocrinology* 2 226 (1940)
3 Young F G *Brit Med Bull* 5 155 (1947)
4 Folley S J and F G Young *XIth Int Congr Chem 1947* 4 77 (1952)
5 Stricker P and F Grueter *C R Soc Biol Paris* 99 1978 (1928)
6 Riddle O Bates R W and S W Dykshorn *Am J Physiol* **105** 191 (1933)
7 Azimov G J and N K Krouze *J Dairy Sci* 20 289 (1937)
8 Folley S J and F G Young *Proc Roy Soc (London)* *B* **126** 45 (1938)

most potent of Professor Landgrebe s preparations gave a detectable response at a concentration of only 1 25 μg /ml (Table 6) a very much lower concentration than the detected minimum for any other of the active preparations studied (Bradley et al [34]) Moreover Mr Bradley s subsequent results indicate a correlation between melanophore expanding potency as measured in Professor Landgrebe s laboratory on *Xenopus*, and the activity on the slope of the composite respiration curve of rat mammary tissue The evidence suggests therefore that intermedin (B ') is particularly active in causing an increase in the slope of the composite respiration curve of lactating rat mammary slices (see Fig 4) and that the activity shown by some other pituitary preparations including somatotropin preparations in relatively high concentrations may be due to contamination with intermedin This is borne out in general by melanophore-expanding assays on a number of these preparations carried out on *Xenopus* in Professor Landgrebe s laboratory

Table 6

EFFECT OF PURIFIED INTERMEDIN (950 I U /MG) ON THE COMPOSITE RESPIRATION CURVE OF LACTATING RAT MAMMARY GLAND SLICES
(from Bradley et al [34])

Rat No	*Day of Lactation*	*Net Gas Evolution as μl CO /100 mg moist tissue/2 hr at 37*		
		Control	*B*	
			2 5 μg /ml	
1	6	25	80	P<0 001
		47	79	
2	7	78	90	
		56	100	
3	9	42	108	
		65	140	
4	9	117	146	
		100	147	
			1 25 μg /ml	
5	6	87	111	0 01>P>0 001
		72	107	
		79	104	
		95	108	

Now in recent unpublished work Mr Bradley has shown that a highly purified intermedin preparation (550 I U /mg) for which we are indebted to Professor F W Landgrebe will cause an increase in the R Q and glucose uptake of lactating rat mammary gland slices metabolising acetate plus glucose There is little doubt that this effect is connected with the ability of intermedin to increase the slope of the composite respiration curve of this

Table 1

EFFECT OF DAILY INJECTIONS OF 100 MG GROWTH HORMONE FOR 14 DAYS

Cow		Milk (lbs.)	Fat (lbs.)	Increase Milk (%)	Increase Fat (%)
Tulip					
100 mg Growth Hormone daily	Initial 3 day ave	36.4	1.34		
	12 13 14 day ave	55.4	2.95	52	120
Lula					
100 mg Growth Hormone daily	Initial 3 day ave	25.8	1.20		
	12 13 14 day ave	40.2	2.89	56	141

per cent during such injection periods One of the studies reported a year ago is shown in Table 1 It will be noted that milk production increased over 50 per cent and fat production over 120 per cent during a 14 day period in which 2 cows in lactation for approximately 2 months received 100 mg of GH daily for 14 days Since the energy intake was not changed



FIG 1 Effect of GH on mammary gland of cow Note gland before (left) and after (right) administration of 100 mg GH daily for 14 days

9 Cotes P M Reid E and F G Young *Nature* (London) **164** 709 (1949)
10 Cotes P M Crichton J A Folley S J and F G Young *Nature* (London) **164** 992 (1949)
11 Flux D S Folley S J and S J Rowland *J Endocrinology* **10** 333 (1954)
12 Shaw J C Chung A C and I Bunding *Endocrinology* (in press 1954)
13 Donker J D and W E Petersen *J Animal Sci* **10** 1074 (1951)
14 Donker J D and W E Petersen *J Dairy Sci* **35** 503 (1952)
15 Chung A C Shaw J C and W M Gill *J Dairy Sci* **36** 589 (1953)
16 Wrenn T R and J F Sykes *J Dairy Sci* **36** 1313 (1953)
17 Brumby P J and J Hancock *New Zealand J Sci Tech* **36** 417 (1955)
18 Jordan R M and D D Shaffhausen *J Animal Sci* **13** 706 (1954)
19 Biavati F and C Fiori *La Nuovo Veterinaria* **26** 363 (1950)
20 Young F G *Recent Progr Hormone Research* **8** 471 (1953)
21 Balmain J H Folley S J and R F Glascock *Biochem J* **56** 234 (1954)
22 Folley S J and T H French *Biochem J* **45** 117 (1949)
23 Folley S J and T H French *Biochem J* **46** 465 (1950)
24 Balmain J H and S J Folley *Biochem J* **49** 663 (1951)
25 Balmain J H Folley S J and R F Glascock *Biochem J* **52** 301 (1952)
26 Balmain J H and S J Folley *Arch Biochem 2nd Biophys* **39** 188 (1952)
27 Balmain J H French T H and S J Folley *Nature* (London) **165** 807 (1950)
28 Balmain J H Folley S J and R F Glascock *Nature* (London) **169** 447 (1952)
29 Balmain J H Cox C P Folley S J and M L McNaught *J Endocrinology* **11** 269 (1954)
30 Milman A E and J A Russell *Endocrinology* **47** 114 (1950)
31 Ottaway J H *Brit Med J* ii 357 (1953)
32 Bulbrook R D and J H Ottaway *J Physiol* **123** 57P (1954)
33 Recant L *J Clin Invest* **31** 656 (1952)
34 Bradley T R Folley S J Landgrebe F W and G M Mitchell *Biochim et Biophys Acta* **13** 449 (1954)

DISCUSSION

Influence of Growth Hormone on the Mammary Gland and on Human Metabolism

Designated Discussion

J C SHAW (University of Maryland College of Agriculture) I should like to present briefly some results from our laboratory of the effect on cows of growth hormone (GH) ACTH TSH and the glucocorticoids under various physiological conditions which have a bearing on the interesting reports of Dr Cole and Dr Folley

We have injected cows in various stages of lactation with from 50 to 100 mg of GH daily for periods varying from a few days to seven weeks and have elicited and maintained increases of milk production of 25 to 50

the same The practical aspects of these observations are readily apparent We may find that we need to breed for high GH production

In another experiment the effect of growth hormone was compared to that of TSH when the latter was given in an amount equivalent to its content as a contaminant in the GH Three groups of 2 cows each were used and the GH and TSH treated groups were reversed at the end of the first period and re treated One set of identical twins was used in this study It will be noted from Table 3 that the milk increasing effect of GH during an 8 day period was quite large whereas that of TSH was but slight In the reversal study which extended over a period of 12 days the same relationship was observed Plasma protein bound iodine was not altered from normal in these studies

Table 3

THE COMPARATIVE GALACTOPOIETIC EFFECTS OF GH AND TSH* ON DAILY MILK PRODUCTION† (3 GROUPS OF 2 COWS EACH WITH ONE SET OF MONOZYGOUS TWINS IN GH AND TSH GROUPS)

	Control (lbs milk)	*GH (lbs milk)*	*TSH (lbs milk)*
(50 mg GH for 5 days + 100 mg for 2 days)			
Initial	23	28	18
Final	25	35	23
(50 mg GH for 6 days + 100 mg for 6 days) GH and TSH groups reversed			
Initial	24	14	18
Final	21	26	19

* Amount injected same as that present in the GH
† Initial milk production was average of 2 days preceding and final was average of last 2 days of injection

In 1952 we reported that a single intramuscular injection of 100 to 300 I U of purified ACTH (Armour) in 3 normal cows increased blood glucose from approximately 45 to over 120 mg per 100 ml and at the same time decreased milk production by over 50 per cent within 36 hours The data shown in Table 4 confirmed the earlier report of Dr Folley and his group who used a partially purified preparation More recently we were able to demonstrate these same effects with hydrocortisone alcohol as will be noted in Table 5 Note that a single injection of 5 g not only increased blood glucose values considerably above 100 mg per 100 ml but also produced a marked decrease in milk production which was still apparent after 16 days The same doses of cortisone acetate and hydrocortisone acetate produce similar effects but of a lesser magnitude probably due in part to different solubilities and rates of absorption

The foregoing observations were the basis for a study in which a group

during the period, it appears that the great increase in milk fat production may have been due in part, to the resultant partial fasting as I shall point out later on In Figure 1 are shown the photographs of the udder of one of the cows, taken at the beginning and end of the injection period The pictures were taken immediately after milking in both cases The udder not only increased greatly in size but became hard and somewhat inflamed and appeared to be identical to that usually observed on the day of parturition Total blood lipids did not change appreciably during the injection period However, there was an increase in phospholipids neutral fat and free fatty acid fraction and a decrease in cholesterol esters

After a period of 2 weeks during which time milk production decreased to about the normal expected level the cows were subjected to a regime of 50 to 75 mg of GH daily for 7 weeks Energy intake was maintained slightly above requirements Milk production was increased markedly and maintained at a high level The production at the end of 7 weeks was still 20 per cent above the initial level after which time it decreased to about the expected normal level following the removal of GH

Table 2

EFFECT OF GROWTH HORMONE PREPARTUM UPON MILK PRODUCTION
(Monozygous Twins)

	Days in Milk	*Milk (lbs)*	*Fat (lbs)*
100 mg GH 9 days prepartum and 16 days postpartum			
Control twin	280	5418	197
GH treated twin	280	7083	233
100 mg GH 23 days prepartum only			
Control twin	30	648	
GH treated twin	30	834	

Studies on 2 sets of identical twins demonstrated that the administration of GH prior to parturition or prior to and following parturition resulted *in a lasting effect after GH was discontinued In one pair* (see Table 2) the control animal produced 5418 lbs of milk in 280 days whereas its identical twin which received 100 mg GH daily for 9 days prepartum and 16 days postpartum produced 7083 lbs of milk in 280 days During the last 30 days the control animal produced 275 lbs of milk whereas the GH treated animal produced 475 lbs showing that the effect of GH continued throughout the lactation period With the second pair of twins GH was administered only in the prepartal period The control animal produced 648 lbs of milk in the first 30 days postpartum whereas its identical twin which received 100 mg GH daily for 23 days prepartum produced 834 lbs during the same period In each pair the daily intake of feed was exactly

there was a small increase in uncorrected milk and a very marked increase in 4 per cent fat corrected milk due to a large increase in milk fat Total 4 per cent fat-corrected milk was almost as great as that observed in experiment I in which GH alone was used A very striking effect was the large increase in blood glucose levels In experiment IV the ACTH was administered alone Both milk and milk fat decreased sharply but the milk fat exhibited a smaller decrease as shown by the higher level of 4 per cent fat-corrected milk Blood glucose concentration also increased as the result of ACTH but did not reach the peaks observed in the ACTH GH experiment

During the various periods blood ketones remained in the normal range

It appears that experiments II and III comprise the first demonstration of the specificity of the galactopoietic effects of GH For example in experiment II during partial fasting GH reversed the downward trend in milk production The ability of GH to prevent the decline in milk production associated with the administration of ACTH provides a second example of this specificity However GH produces this effect its role appears to be more than "permissive in terms of the concept of Ingle

In view of the discussions which were stimulated by Dr De Bodo s paper I would like to call your attention to experiment III in which we observed very little change in the pounds of milk recovered i e in terms of energy content, but in which there was a marked change in milk composition and a striking elevation in blood glucose levels I believe this experiment may provide a meeting ground where certain issues of the previous discussions may be interpreted

The depression of milk production by ACTH and the glucocorticoid may be due to the well known catabolic effects of ACTH operating in the cow to depress net protein synthesis (milk) in the mammary gland and thus to interfere with milk secretion

In connection with the effects of the various hormone treatments on the concentration of blood glucose the cow apparently is another species in which there is a unique preferential sensitivity or responsiveness to the pituitary endocrine factors regulating carbohydrate metabolism The cow and presumably man are more susceptible to the diabetogenic effect of ACTH than to this effect of GH The cat dog and pig are less resistant to the diabetogenic effects of GH than to that of ACTH The rat on the other hand while susceptible to ACTH appears to be relatively more resistant to the diabetogenic effects of both ACTH and GH In recent studies in our laboratories we have observed that the normal glucose tolerance of cows is not altered appreciably by GH This marked difference between the cat and the cow in respect to their sensitivities to the diabetogenic action of pituitary extracts or growth hormone was not generally known when attention was first directed to the possibility that the diabetogenic action and the galactopoietic action were due to identical substances It is perhaps fortunate

of 6 cows in various stages of lactation, were subjected to a series of experimental conditions In successive periods these were the administration of GH for 8 days partial fasting for 8 days plus GH during the last 5 days of the fast GH and ACTH for 6 days and ACTH alone for 6 days The cows received 110 per cent of their calculated energy requirements throughout the experiments with the exception of the fasting period during which time they received 40 per cent of their requirements A rest period of 12 to 15 days followed each treatment period

Table 4

EFFECT OF ACTH ON BLOOD GLUCOSE AND MILK PRODUCTION ON NORMAL COWS

Cow	I U of ACTH	Hours			
		0	24	48	96
		Mg /100 ml blood glucose			
1	100	44 7	131 6	42 7	—
2	200	45 5	126 9	45 5	—
3	300	46 0	123	58 5	—
		Lbs milk daily			
1	100	26 4	12 0	20 2	25 8
2	200	49 6	38 5	45 5	47 6
3	300	53 1	18 4	29 6	36 0

Table 5

EFFECT OF INTRAMUSCULAR INJECTIONS OF 5 GRAMS OF HYDROCORTISONE ALCOHOL ON BLOOD GLUCOSE AND MILK PRODUCTION

Cow		Days after Injection				
		0	1	2	16	20
1	g*	56 0	121 0	87 5	—	45 7
	m†	26 3	13 4	9 8	21 1	—
2	g	41 5	114 3	76 4	—	48 3
	m	45 8	32 5	28 6	36 5	—

* g—blood glucose in mg 100 ml
† m—daily milk production in lbs

Milk and milk fat increased uniformly during the period of GH injection (experiment I) and blood glucose increased slightly During the first 3 days of the fasting period (experiment II) milk production and blood glucose both decreased GH corrected the downward trend of both producing some increase in blood glucose an increase in milk production and an even greater increase in milk fat During the ACTH GH period (experiment III)

pairs of identical twins received an equivalent of amount of thyrotropic hormone They observed a very definite stimulation of lactation over the 8 week period during which the TSH was administered Accordingly the effects of the so called growth hormone I believe under both types of treatment should be considered due in part, to the thyrotropic contaminant This might have some bearing on the pre parturiant effects of growth hormone and possibly might explain why early postpartum lactation might be affected Dr Shaw should extend his observations on cows i e investigating further the pre parturiant effects of these hormones I believe there was only a single cow included in the studies It is a point which should be further explored

JOSEPH MEITES (Michigan State College) We have been doing some work with growth hormone in goats and I have shown these results to Dr Folley when he recently visited our campus Unlike his experience we were able to obtain increases in milk production with growth hormone We have also studied the effects of cortisone at levels of 100 mg per goat per day These goats weighed from 80 to 120 pounds each Cortisone was administered for 5 days and after stopping for one week it was given again We achieved absolutely no reduction in milk secretion Of course it is quite possible that larger doses of cortisone might have produced a reduction in milk secretion

I would like to say also in connection with growth hormone that these goats had been receiving Protamone® a thyroid active material It is very characteristic of thyroid treated dairy cows or goats that when the thyroid material is stopped there is a very pronounced depression in lactation Sometimes it drops to nothing and then gradually returns to a limited extent In Figure 2 it can be seen that the marked decrease in milk production which occurred in the controls was partially prevented by the growth hormone injections This is interpreted to suggest that part of the galacto poietic action of Protamone® and other thyroid active substances is mediated through an increased secretion of growth hormone by the pituitary

Now some contradictory results or seemingly contradictory results were obtained in rabbits which we treated with growth hormone Mature or almost mature female rabbits weighing to 6 to 8 pounds were castrated and treated for 25 days with estrone and progesterone to first develop their mammary glands We then gave some of them prolactin alone for 8 days while others received prolactin and growth hormone The growth hormone was an Armour preparation and kindly supplied by Dr Bunding The rabbits which received the prolactin alone showed a very intense lactation The rabbits which received the prolactin and growth hormone showed a very marked inhibition of lactation Not only was there very little milk but the mammary glands of these rabbits were actually quite shrunken We are now conducting some metabolic studies in rabbits and it was very informa

since it has been clearly shown that GH is not appreciably diabetogenic in the cow If our observations are correct that GH given during the pre parturient period exerts a marked influence on milk production during the entire lactation period we may have to conclude that the term galacto poietic does not adequately describe the functions of GH in relation to the mammary gland

The action of GH on lactation may be that of increasing the availability of milk precursors in the blood increasing the efficiency of milk secretion or, actually producing an increase in the growth of mammary tissue

Our failure to demonstrate a ketogenic effect of GH on normal and fasted cows is perhaps not surprising in view of the fact that the cow and other ruminants by virtue of their peculiar digestive system, utilize short chain fatty acids and that the tissues are probably conditioned to the utilization of greater amounts of ketone bodies This is true at least for the mammary gland since we have shown that β hydroxybutyrate is one of the major metabolites of the active gland We observed the incorporation of relatively large amounts of C_{14} labeled β hydroxybutyrate into the short chain fatty acids of milk fat in bovine mammary gland perfusion studies A competition was also demonstrated between acetate and β hydroxybutyrate in that the perfused bovine gland showed a preference for acetate

I should like to submit that in the lactating mammary gland of the ruminant we have an excellent organ for the study of tissue elaboration (if you wish to consider milk as a tissue) and that in this tissue the responses to various treatments are easily measured without destroying the organ in which the elaboration is indeed great Likewise this is an excellent organ for arteriovenous studies since it is virtually isolated anatomically from the body

General Discussion

C W TURNER (University of Missouri) I have been very greatly interested in the papers presented this afternoon In connection with the first paper that of Dr Cole I might say that we have followed similar plans of hormone treatment in our investigations I think the only point of departure is our belief that progesterone is not necessary when the pituitary lactogenic frac tion is administered In other words we have been able to obtain more or less full growth of the mammary gland of the hypophysectomized animal receiving estrogen and the lactogenic fraction So in that connection we feel that they should attempt the elimination of progesterone from the combined treatment

In respect to the second paper I wish only to call attention to one point This pertains to the investigative work done in New Zealand using the growth hormone in identical twins This growth hormone which was used possibly by Dr Shaw had a considerable contamination with TSH and to compensate for that contamination they also conducted a trial in which

tive to hear Dr Milman indicate yesterday that at least in young rabbits small doses of growth hormone will induce an intense diabetogenesis and an intense glycosuria If this had been true for our rabbits it could very well account for the depressing effect of growth hormone on lactation

Finally I think there is one thing which warrants emphasis a point which both Dr Cole and Dr Shaw mentioned It is that we must recognize the necessity of at least three hormones for lactation mainly prolactin growth hormone and hydrocortisone Of course Dr Folley has also stressed this a good deal in the hypophysectomized animals If we think in terms of what an intact animal needs for the initiation of lactation then there may be some question as to whether any hormone but prolactin is actually needed in extra amounts I say this because in all animals which have been studied with the possible exception of the rat it is possible to inject prolactin alone and initiate lactation This has been done in dogs rabbits guinea pigs monkeys and in many other species I think this point might bear some comment from the three gentlemen who have spoken on the program

P J RANDLE I just want to share a few results Professor Young asked me to mention which relate to further studies on the galactopoietic effect of growth hormone under the conditions which Dr Folley has already described We have observed that there is a reasonable linear relationship between the percentage increase of milk yield and the dose of growth hormone administered We have been unable as yet to dissociate or distinguish between the growth promoting and the galactopoietic activities of different preparations of growth hormone In further preliminary experiments Dr McCormick and Professor Young have obtained similar evidence of a galactopoietic action from small doses of insulin something in the order of 5 units given to an intact cow

OSCAR RIDDLE (Chairman) I wish to make a few brief remarks concerning the paper of Dr Folley We understood that the early result which indicated prolactin affected the respiratory curve the uptake of oxygen now appeared to be vitiated by later work in which it was found that intermedin was playing the large part That strikes me as being an exceedingly unusual and unexpected result Prolactin by its method of preparation is I think the one pituitary hormone which is easiest to free of intermedin at least by the methods which we have always used and which I am familiar with We sent three preparations of prolactin and a series of other pituitary preparations to Dr Geiling for assay of intermedin The prolactin preparations were entirely free They were the only preparations from the pituitary which were free of intermedin so Dr Folley s finding seem to be a rather remarkable result

Secondly Dr Folley stated that the pituitary lactogenic hormone prolactin was discovered by Stricker and Grueter They discovered an *effect*

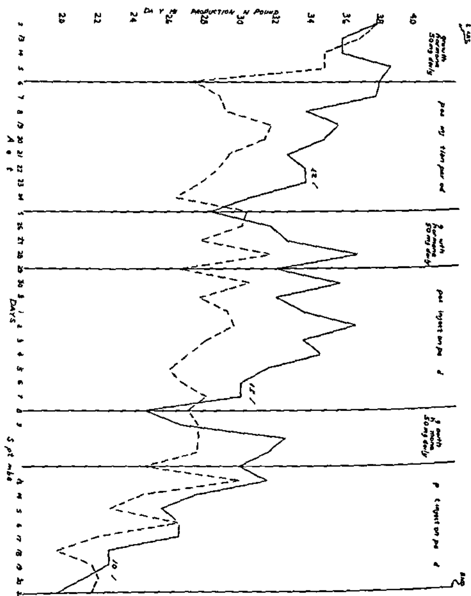


FIG 2 Effects of growth hormone (Merck) on daily milk production in goats The solid line above represents data from observations on 4 goats which received growth hormone The stippled line represents data from 4 control animals The figures above the solid line indicate the total percentage increase in milk yields during each injection and post injection period Just prior to the start of the experiment all goats had been fed Protamone® (iodinated casein) for about 3 months

powerful galactopoietic agent, certainly in the cow under suitable conditions

I was very cheered to hear from Dr Meites that he obtained a positive galactopoietic effect of growth hormone in goats because we have invested quite heavily in establishing a goat herd in our department I was beginning to wonder whether the goat was a suitable animal for experiments of this nature

With respect to the role of the adrenal cortex in lactogenesis it has been shown clearly by Reece and collaborators that prolactin does not very effectively induce lactation in the pseudopregnant normal rat but when it is given in combination with the corticoids or ACTH then one observes a positive effect

OSCAR RIDDLE In regard to the intermedin I have just one further word We never could separate completely thyrotropin from gonadotropin Those two substances together were the ones that contained the extremely high intermedin values whereas none could be demonstrated by Geiling s laboratory in the prolactin preparations submitted

ROGER D COLE As long as we are talking about the intermedin problem I might mention that we have on hand some prolactin which is remarkably free of intermedin and which we shall be glad to send to you Dr Folley, if you would like to check this It is also free of other pituitary factors

I think Dr Folley has very adequately answered most of the questions raised except one which was raised by Dr Turner regarding the essentiality of progesterone in mammary development Now he mentioned that the animals he used were hypophysectomized but he did not specify whether or not they were gonadectomized Since he has left the auditorium we can't settle this question I suggest however that this might be the answer to the problem

O H PEARSON (Sloan Kettering Institute for Cancer Research) I would like to present a single observation which indicates that a growth hormone preparation (beef) supplied by the Armour Laboratories induced a positive effect in human breast cancer We have studied three patients with metatastic mammary cancer in whom the disease was in relapse after castration and adrenalectomy and who obtained remissions from hypophysectomy One of these patients has been in remission 14 months after hypophysectomy At the third month following hypophysectomy we administered growth hormone to determine if we could stimulate the growth of the tumor In Figure 3 are shown data from this patient She had an osteolytic tumor and as the tumor grew it destroyed bone We indirectly measured the growth of the tumor by the rate of bone destruction determined from urinary calcium In the control periods the urinary calcium excretion was

of a pituitary hormone In 1927 even before their result we had also observed the effect of something in the pituitary which caused the crop gland of the pigeon to enlarge but we did not discover the lactogenic hormone or prolactin in 1927 It was 5 years later before we could discover the hormone *We had to find that the crop gland response to the pituitary* extract was not caused by growth hormone by either of the two gonadotropins or by thyrotropin all of which were known then adrenocorticotropin was just coming along It was only after such a survey that a hormone could be declared The responses to prolactin have now become quite variable such as lactogenesis crop gland increase, maternal behavior, antigonadal response and in some animals the raising of the blood sugar or diabetogenesis But, these are *responses* they are not hormones, of course

T LEVITT Could the three speakers clear up one small point? Drs Lyons and Cole stated quite categorically that, in their work with animals thyroid hormone produces no effect on mammary growth Dr Folley believes that it has indirect actions on milk production in cows Dr Shaw stated quite categorically that thyrotropic hormone and I believe thyroid hormone definitely increase milk production Could they elucidate this point and tell us whether it may apply to man?

S J FOLLEY I would first like to apologize to the chairman I certainly did not intend to minimize the important work on the isolation of prolactin *which he and his collaborators did and for which we are all grateful* I should have said in respect to the 1928 work of Stricker and Grueter that they had discovered a lactogenic effect in pituitary extracts The isolation of a hormone as the chairman pointed out came from his laboratory a few years later

With regard to his point about intermedin in prolactin preparations all I can say is that the preparations have been assayed for intermedin by Professor Landgrebe on *Xenopus* and he has found intermedin by that test in all our preparations

Dr Turner mentioned the New Zealand experiments He said that they had carried out parallel experiments with TSH using a dose equivalent to the amount present in the growth hormone they had administered As I indicated in my presentation I had seen the New Zealand paper—they kindly sent me a copy of it—and my interpretation of their results is that they had excluded TSH contamination from having played any great part in their results

With regard to the question which Dr Levitt had asked us about thyroid I can only reply that we have never been able to show any synergistic effect of thyroid in mammary growth experiments It is quite well known however, that either thyroid hormone L thyroxin or tri iodothyronine is a

powerful galactopoietic agent certainly in the cow under suitable conditions

I was very cheered to hear from Dr Meites that he obtained a positive galactopoietic effect of growth hormone in goats because we have invested quite heavily in establishing a goat herd in our department I was beginning to wonder whether the goat was a suitable animal for experiments of this nature

With respect to the role of the adrenal cortex in lactogenesis it has been shown clearly by Reece and collaborators that prolactin does not very effectively induce lactation in the pseudopregnant normal rat but when it is given in combination with the corticoids or ACTH then one observes a positive effect

OSCAR RIDDLE In regard to the intermedin I have just one further word We never could separate completely thyrotropin from gonadotropin Those two substances together were the ones that contained the extremely high intermedin values whereas none could be demonstrated by Geiling s laboratory in the prolactin preparations submitted

ROGER D COLE As long as we are talking about the intermedin problem I might mention that we have on hand some prolactin which is remarkably free of intermedin and which we shall be glad to send to you Dr Folley if you would like to check this It is also free of other pituitary factors

I think Dr Folley has very adequately answered most of the questions raised except one which was raised by Dr Turner regarding the essentiality of progesterone in mammary development Now he mentioned that the animals he used were hypophysectomized but he did not specify whether or not they were gonadectomized Since he has left the auditorium we can t settle this question I suggest however that this might be the answer to the problem

O H PEARSON (Sloan Kettering Institute for Cancer Research) I would like to present a single observation which indicates that a growth hormone preparation (beef) supplied by the Armour Laboratories induced a positive effect in human breast cancer We have studied three patients with metatastic mammary cancer in whom the disease was in relapse after castration and adrenalectomy and who obtained remissions from hypophysectomy One of these patients has been in remission 14 months after hypophysectomy At the third month following hypophysectomy we administered growth hormone to determine if we could stimulate the growth of the tumor In Figure 3 are shown data from this patient She had an osteolytic tumor and as the tumor grew, it destroyed bone We indirectly measured the growth of the tumor by the rate of bone destruction determined from urinary calcium In the control periods the urinary calcium excretion was

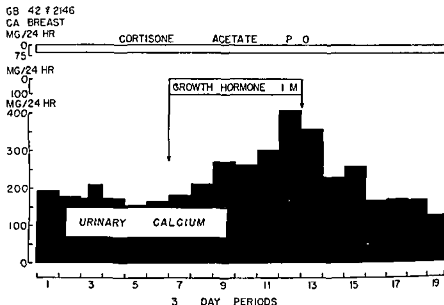


FIG 3

at the upper limits of normal indicating the patient was in remission When growth hormone was given there was a step wise rise in calcium excretion which subsided when the growth hormone was withdrawn In Figure 4 is shown the complete balance of nitrogen phosphorus and calcium You will note that there was a negative phosphorus and calcium balance the loss of calcium and phosphorus being in the same proportion as these ions exist in bone If there was any change a slightly negative nitrogen balance re sulted

We can conclude from this experiment that growth hormone induced osteolysis in this patient which effect we interpret to be due to an increased growth of the tumor At a later stage this patient was given TSH which induced some stimulation of thyroid function but it failed to have any effect on calcium excretion Further observations are obviously necessary

J FURTH (Children s Cancer Research Foundation Inc) The prevalent procedure used in analyzing the complex deficiency created by hypophy sectomy is based on chemical attempts to isolate different substances from the pituitary There are other possibilities The one explored by us* con sists of separating the pituitary into its morphologic units that is into as many functional cell types as this organ possesses This can be done by producing monomorphous hormone secreting pituitary tumors either by a carcinogen as ionizing irradiation or by a specific deficiency of one pituitary hormone left uncompensated over very long periods of time (more

* These investigations are being supported by Grant C 2259 United States Public Health Services

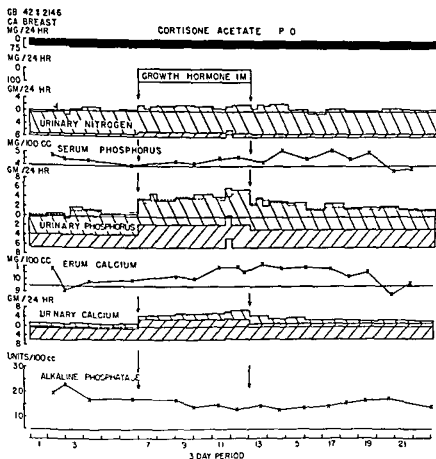


Fig 4

than half of the life span of the animal) The latter results in a compensatory tumor like hyperplasia of the corresponding tropic cell and this cell mass can be grafted on histocompatible hosts with a similar target hormone deficiency

The tumors which have been best studied are thyrotropic and these have been induced by destruction or removal of the thyroid or blockage of synthesis of the thyroid hormone Next well known are the adrenotropic tumors of which three strains were isolated They are essentially alike all causing a marked obesity hypertrophy of the adrenal (Figs 5 6) without any stimulation of other endocrine glands involution of the thymus atrophy of the spleen and lymph nodes extraordinary sensitivity to endogenous saprophytes polydypsia and polyuria (with about 10 times the normal urine output) All these changes can be reversed by adrenalectomy which results in a specific enhancement of the growth of these adrenotropic tumors These tumor cells are not only devoid of other types of secretions but are

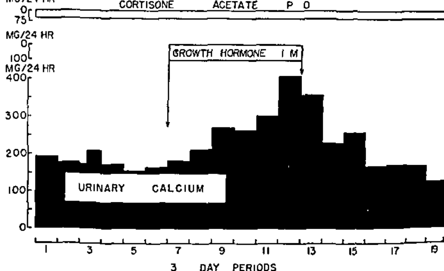


FIG 3

at the upper limits of normal indicating the patient was in remission When growth hormone was given there was a step wise rise in calcium excretion which subsided when the growth hormone was withdrawn In Figure 4 is shown the complete balance of nitrogen phosphorus and calcium You will note that there was a negative phosphorus and calcium balance the loss of calcium and phosphorus being in the same proportion as these ions exist in bone If there was any change a slightly negative nitrogen balance resulted

We can conclude from this experiment that growth hormone induced osteolysis in this patient which effect we interpret to be due to an increased growth of the tumor At a later stage this patient was given TSH which induced some stimulation of thyroid function but it failed to have any effect on calcium excretion Further observations are obviously necessary

J FURTH (Children s Cancer Research Foundation Inc) The prevalent procedure used in analyzing the complex deficiency created by hypophysectomy is based on chemical attempts to isolate different substances from the pituitary There are other possibilities The one explored by us* consists of separating the pituitary into its morphologic units that is into as many functional cell types as this organ possesses This can be done by producing monomorphous hormone secreting pituitary tumors either by a carcinogen as ionizing irradiation or by a specific deficiency of one pituitary hormone left uncompensated over very long periods of time (more

* These investigations are being supported by Grant C 2259 United States Public Health Services

unable to compensate for them in hypohysectomized hosts (Experiments with Dr E Anderson and R C Bohn)

One transplantable tumor (Strain 7) causes a marked somatotropic combined with some thyrotropic, stimulation The weight of these tumor bearing animals increases by about eighty per cent (Fig 7) The liver and heart weights are nearly doubled In the liver there is a tremendous increase in size of parenchymatous cells which increase envolves both cytoplasms

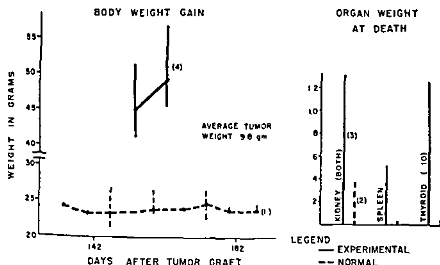


FIG 7 The gain in total body kidney spleen and thyroid weights of animals bearing grafted complex pituitary tumors (Strain 7) The numbers in parenthesis indicate the number of animals in the respective groups The vertical lines show the range of body weights the connecting lines the means The increase of liver weights of tumor bearing animals is of about the same order of magnitude as that of kidney weights

and nuclei There is a marked increase of basophilia indicating greatly enhanced DNA and RNA synthesis there are also many mitotic figures indicating true growth (Figs 8 9) The size of the spleen is increased by 5 to 10 fold this is due primarily to erythropoiesis as Dr Dougherty described in animals receiving growth hormone The microscopic appearance of the spleen does not suggest sequestration of erythrocytes None of the animals bearing different types of transplantable pituitary tumors have such greatly enlarged spleens There is an associated extensive hyperplasia of the bone marrow evidence of erythrocyte destruction is wanting Thus it seems that the growth hormone in question stimulates erythropoiesis Unlike the animals bearing corticotropic tumors the hosts of somatotropic tumors are

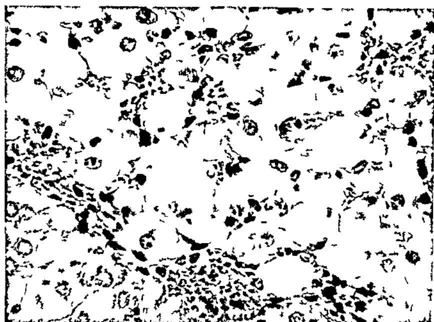


FIG 5 The adrenal cortex and small part of the medulla (left lower corner) of a mouse bearing an adrenotropic tumor (×450)

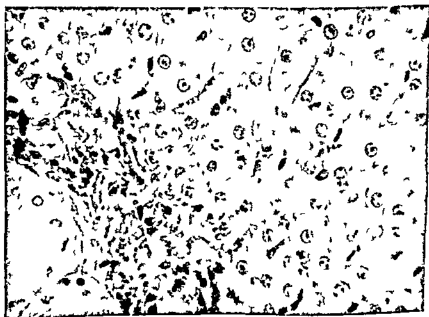


FIG 6 The same area of the adrenal cortex of a normal mouse shown at same magnification as that of Figure 5 (×450)

not obese The mammary glands are slightly stimulated Homologous anabolic hormone is certainly not toxic Animals bearing such tumors are large but as healthy and active as an animal bearing any transplantable tumor that I have studied Consequently the toxic changes by growth hormone used in an alien species and reported yesterday are I believe a foreign protein intoxication



Fig 10 The characteristic microscopic appearance of a lacto-somatotropic tumor These tumor cells are either chromophobic or have coarse acidophilic granules (×112 5)

The most recent members of our transplantable pituitary tumor series are mammotropic (Fig 10) Several such strains are available Common to all is a ducto-alveolar stimulation of the mammary gland with secretion (Figures 11 12) None stimulates the thyroid gland gonads or adrenals but some also have somatotropic properties with liver (Fig 8) and spleen (Fig 13) changes as described for the growth hormone They tend to be acidophilic and the newest member (Strain 8) is markedly acidophilic The observations thus far point to some relation between somatotropic and mammotropic effects but also indicate the independence of the respective functions

Dr Houssay informed me that he has produced gonadotropic tumors in the rat by gonadectomy and is now at the stage of transplanting them Thus in the near future almost all types of hormone secreting pituitary tumors will be available It is evident that these monomorphous and in part monohormonal tumors offer new possibilities for the isolation of pure

FIG 8 The microscopic appearance of the liver of a mouse bearing a lactosomatotropic tumor (Strain 4) There is disseminated focal hypertrophy of liver cells with intervening liver cells of approximately normal size This represents an incipient or mild anabolic stimulation in the liver while Figure 9 illustrates advanced anabolic stimulation (×112 5)

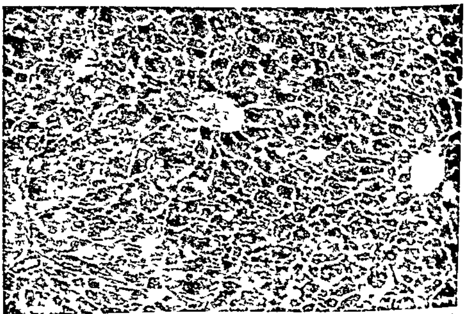


FIG 9 Advanced diffuse hypertrophy of liver cells of a mouse bearing complex tumor (Strain 7) Note the great enlargement of liver cells both nuclei and cytoplasms the mitotic figures and the increased basophilia of the cytoplasm (×112 5)

Fig 12 Ducto-alveolar hyperplasia of the mammary gland in the inguinal region with small part of adjacent inguinal lymph node of a mouse bearing a lacto somatotropic tumor graft

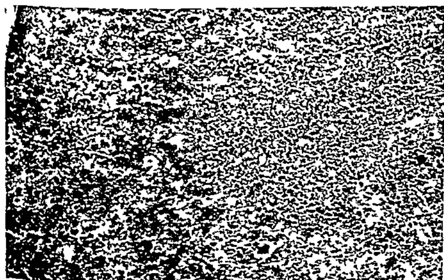


Fig 13 The spleen of a mouse bearing a lacto-somatotropic tumor showing moderate erythropoietic activity with many megakaryocytes (×112 5)



FIG 11 Ducto alveolar hyperplasia of a mouse bearing a small grafted lacto-somatotropic pituitary tumor

pituitary hormones and for the study of fundamental physiologic problems such as those reported here during the past few days

I am optimistic Dr Fell with respect to the possibilities of growing such monomorphous tumors *in vitro* The problem is to provide the specific physiologic stimulants and to remove the physiologic antagonists, e g attempted growth of thyrotropes calls for the plasma of athyroid animals If the source is human hormone secreting tumor human hormonal proteins will be produced

EDWIN A MIRAND (Roswell Park Memorial Institute) Little attention has been paid to the possible influence of growth hormone on tumor growth In our studies with mice bearing transplantable mouse tumors particularly adenocarcinoma of the breast we have shown consistently that the administration of growth hormone to these animals will cause an increase in the growth constant a decrease of 20–25 per cent in the cell number doubling time or mean intermitotic time and a significant decrease in the tumor latent period The foregoing effects of growth hormone on the growth con

28

Human Studies with Purified Pituitary Growth Hormone Preparations *

Laurance W Kinsell
Institute for Metabolic Research of the Highland Alameda County Hospital
Oakland California

Our experience with pituitary growth hormone evaluation in human subjects dates back to 1948 At that time using material prepared by Dr Li we carried out balance studies in three normal young adult males under controlled metabolic ward conditions In all three pyrexia and malaise appeared during the administration of the pituitary growth hormone and metabolically there was a net loss rather than a net retention of nitrogen and other constituents of protoplasm (Fig 1) It was concluded that the observed effects were consistent with those which would result from the administration of many pyrogenic foreign proteins and that if any true growth hormone were present its metabolic effects were completely masked

In 1949–1950 studies using pituitary growth hormone prepared by Drs Hays and Steelman and their associates of the Armour Laboratories also failed to produce any metabolic effects which could be construed as being compatible with growth promotion (Fig 2) In these studies as well as those carried out with Dr Li s material the pituitary derivative under investigation was administered intramuscularly

In 1952 a study was carried out using one of the same preparations which had been evaluated in 1950 There were three significant changes in the metabolic program at that time the *first* being that a patient with no thyroid tissue was the subject chosen the *second* that the material was administered by continuous intravenous drip rather than by intramuscular administration and the *third* difference lay in the fact that the patient was

* These studies have been carried out in association with Drs George D Michaels Nadine Foreman Harry E Balch and with the technical assistance of Etsuko Osawa June Bilisoly Marjorie Coelho Florence Olson and George Fukayama

stant, intermitotic time and tumor latent period, as well as the increase on body weight and organs of these animals can be counteracted by the administration of ACTH or cortisone The antagonistic action of ACTH to growth hormone was further demonstrated in hypophysectomized rats This similar antagonistic effect is not seen in adrenalectomized rats or adrenalectomized mice with transplantable tumors This antagonism of ACTH and cortisone to growth hormone might explain the conflicting results of some studies using growth hormone particularly preparations containing adequate traces of ACTH

Studies were carried out to determine whether growth hormone when administered to fasted mice with transplantable tumors could still influence tumor growth Preliminary studies show that growth hormone does have a stimulating effect on the growth constant intermitotic time and tumor latent period It seems that growth hormone can produce effects independent of caloric intake

FIG 2 Lack of significant effect of "pituitary growth hormone on nitrogen balance or body weight either alone or in conjunction with ACTH No pyrexia occurred In contrast the anabolic effects of testosterone are apparent (1949–1950 studies) Negative calcium and phosphorus balances also were noted during pituitary growth hormone administration

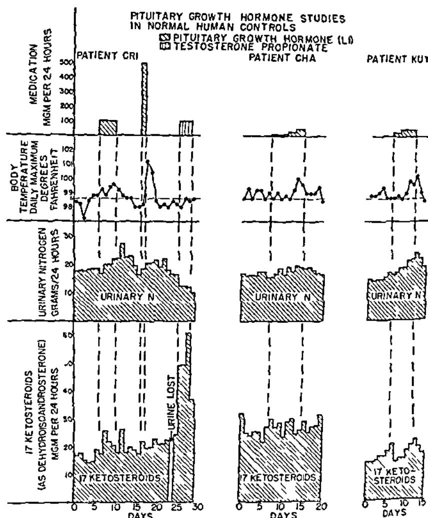


FIG 1 Hyperpyrexia and increased nitrogen excretion in 3 normal male patients during administration of pituitary growth hormone (1948 studies) Hyperglycemia resulted in Patient CRI during the administration of 500 mg of pituitary growth hormone per day Testosterone produced its characteristic anabolic response in this patient

maintained on a synthetic formula diet It was made up entirely of protein and fat supplemented with essential minerals and vitamins whereas previous studies had been carried out with patients maintained on a mixed diet A significant growth effect was obtained in terms of chemical changes (retention of nitrogen potassium and phosphorus) and in terms of weight gain (Fig 3)

Whether the effects obtained in this patient were primarily attributable to the lack of thyroid tissue or to the intravenous administration of the pituitary preparation and whether the unusual nature of the diet might have been of any significance it is impossible to say at the present time

Later in 1952 other growth hormone preparations were administered intravenously to other patients including materials prepared by Dr Raben and his associates and materials obtained from Dr Steelman and his associates (Armour Laboratories) who used the Raben technique in the preparation The results of these studies are shown in Figures 4 A B C

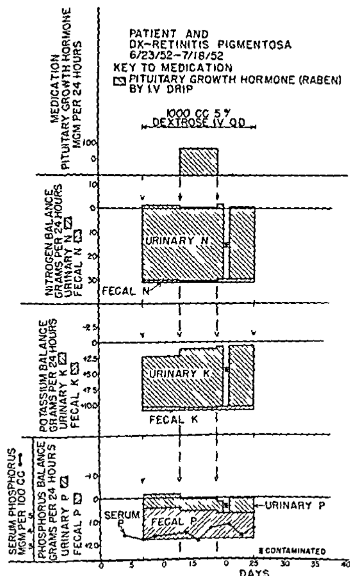


FIG 4A Lack of consistent changes in constituents of protein tissue in a patient receiving pituitary growth hormone" (Raben) No effect on circulating eosinophils or urinary 17 ketosteroids was observed

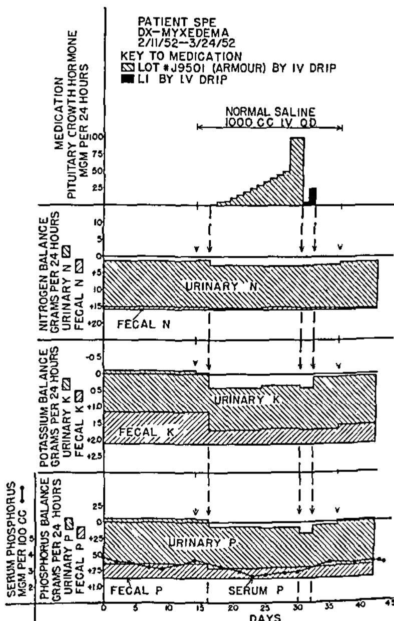


FIG 3 Apparent retention of nitrogen potassium and phosphorus during administration of pituitary growth hormone to a patient with myxedema There was a gain in weight during the pretreatment control period (3 pounds) and during treatment with Armour s pituitary growth hormone (9 pounds) A weight loss of 4 pounds occurred following the omission of Armour s pituitary growth hormone

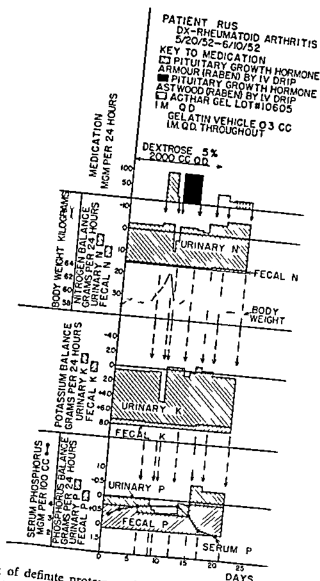


FIG 4C Lack of definite protein anabolic effect from either of two growth hormone preparations (pituitary growth hormone Armour Lot #491082 and Raben s) Both patients receiving the Raben material retained phosphorus Calcium determinations unfortunately were not carried out Eosinophil and 17 ketosteroid changes marked fluid retention and amelioration of arthritis in this patient during administration of pituitary growth hormone (Armour Lot #491082) indicated its high ACTH content Lack of potassium loss *may* have been referable to its pituitary growth hormone content Pituitary growth hormone (Raben) produced no interpretable effect in the dosage used

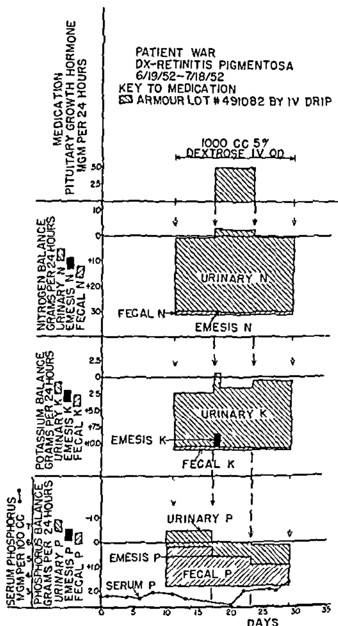


FIG 4B Lack of nitrogen retention in patient receiving pituitary growth hormone (Armour Lot #491082 contaminated with ACTH) A typical ACTH type fall in eosinophils and a rise in urinary 17 ketosteroids were observed

have occurred in others One lot of growth hormone with a low animal assay for thyrotropin on the second day of administration produced major tenderness in the neck By the third day the tenderness was intolerable and was limited precisely to the outline of the thyroid gland The tenderness had disappeared by the third day following discontinuance of hormone administration

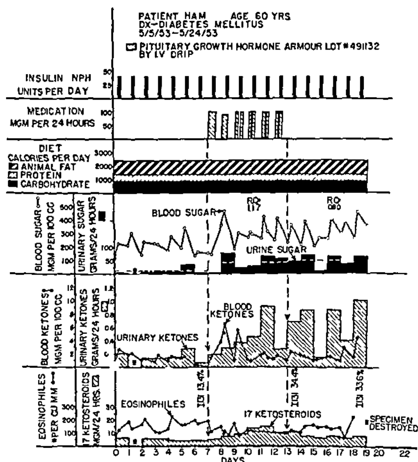


Fig 6A Diabetogenic effects of pituitary growth hormone in a human diabetic

One metabolic study has been carried out in a patient with diabetes of moderate degree Significant accentuation of the diabetic state occurred in terms of increased glycosuria During this period of increased urinary sugar there was negligible change in the excretion of urinary nitrogen A moderate increase in 17 ketosteroids little change in eosinophils and a significant increase in iodine uptake indicated some thyrotropin and probably slight gonadotropin contamination (Figs 6 A B) The total changes would sug

With neither preparation was there any change which could be called a significant protein anabolic effect In the case of the Armour material there was obvious and major ACTH contamination which conceivably may have obscured other effects of co existing hormonal materials

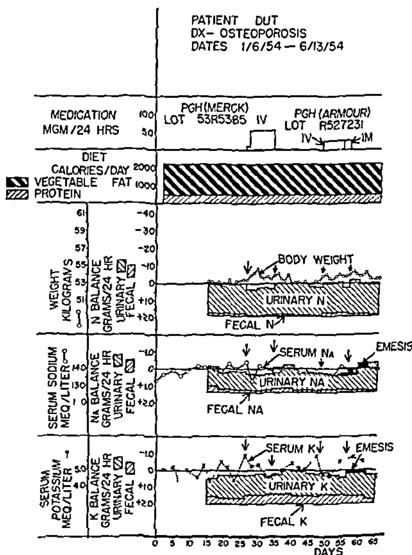


FIG 5 Nitrogen retention and modest weight gain with pituitary growth hormone (Merck Lot 53R5385) administered by i v drip

Several studies have been carried out during the period 1953–1954 using different lots of hormonal material obtained from a variety of sources *Only* one of them that prepared by Drs Folkers and Brink of the Merck Institute, has shown a fairly satisfactory and uncomplicated anabolic effect (Fig 5) Pyrexia has appeared in some individuals and allergic reactions

Discussion

On the basis of the work carried out to date with pituitary growth hormone one might wonder at least insofar as the human is concerned whether a true pituitary growth factor actually exists He need only observe the unfortunate individual with acromegaly (or gigantism) associated with an eosinophilic adenoma or hyperplasia of the anterior pituitary to be assured that there *is* a growth promoting material of pituitary origin which exerts a profound and apparently unlimited anabolic effect in the human subject

Some major questions require answers in regard to growth hormone with particular reference to man

1 What is the normal role of growth hormone?

2 Is one correct in the assumption that a *single* hormonal entity when isolated and available in adequate amount will produce a potent anabolic effect, as will testosterone and other anabolic steroid hormones or may it be that in order for growth hormone to produce its overall anabolic effect a favorable hormonal climate must be present i e certain other hormones must be supplied simultaneously in proper amounts?

3 May species differences account in part for the difficulties so far encountered?

4 Are pituitary growth hormone and the non ACTH pituitary diabetogenic hormone identical or separate?

Our own thoughts regarding the answer to question one is shown in Figure 7 On the basis of observations in normally growing children and in pituitary dwarfs it seems probable that pituitary growth factor is vitally concerned with normal growth prior to puberty and that the pubertal growth spurt is attributable largely to the steroid growth factor There is some evidence suggesting that other factors being equal the level of the inorganic serum phosphorus serves as an index to pituitary growth hormone production and/or effect The phosphorus level is high prior to puberty falls to the normal level during puberty and can be brought to normal in acromegalics and giants by the administration of anabolic steroids This then may be interpreted as indicating that with the onset of puberty the production of pituitary growth hormone is inhibited to a significant degree thereafter The fact that no significant elevation of serum phosphorus occurs in the female after the menopause might suggest that its production is not resumed in significant amounts at this time One might add that the progression of senescence is even more suggestive of such an interpretation Dr Hamilton tells us that in young adult males following castration the serum phosphorus level promptly increases[1] This suggests a post castration increase in pituitary growth hormone production in young subjects

Regarding question two it would seem most unlikely to us that multiple

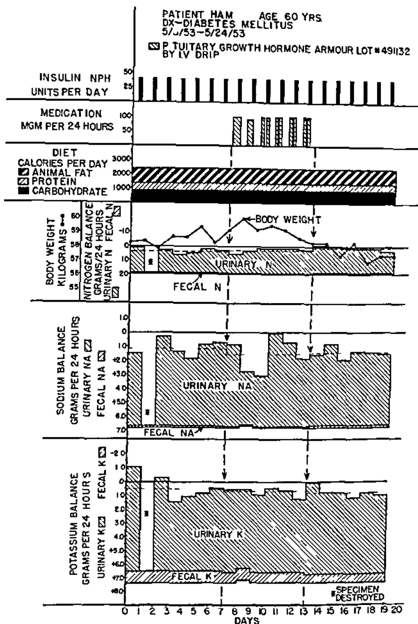


FIG 6B Effects of pituitary growth hormone upon nitrogen sodium and potassium balances in a human diabetic

gest that the diabetogenic effects were attributable chiefly to a pituitary factor other than ACTH and that the growth hormone content of the material was sufficient to inhibit increased protein catabolism which should otherwise have occurred as a result of the increased loss of sugar in the urine Additional studies are underway using pituitary growth hormone without co-administration of insulin

Discussion

On the basis of the work carried out to date with pituitary growth hor mone one might wonder at least insofar as the human is concerned whether a true pituitary growth factor actually exists He need only observe the unfortunate individual with acromegaly (or gigantism) associated with an eosinophilic adenoma or hyperplasia of the anterior pituitary to be assured that there *is* a growth promoting material of pituitary origin which exerts a profound and apparently unlimited anabolic effect in the human subject

Some major questions require answers in regard to growth hormone with particular reference to man

1 What is the normal role of growth hormone?

2 Is one correct in the assumption that a *single* hormonal entity when isolated and available in adequate amount will produce a potent anabolic effect as will testosterone and other anabolic steroid hormones or may it be that in order for growth hormone to produce its overall anabolic effect a favorable hormonal climate must be present i e certain other hor mones must be supplied simultaneously in proper amounts?

3 May species differences account in part for the difficulties so far encountered?

4 Are pituitary growth hormone and the non ACTH pituitary diabeto genic hormone identical or separate?

Our own thoughts regarding the answer to question one is shown in Figure 7 On the basis of observations in normally growing children and in pituitary dwarfs it seems probable that pituitary growth factor is vitally concerned with normal growth prior to puberty and that the pubertal growth spurt is attributable largely to the steroid growth factor There is some evidence suggesting that other factors being equal the level of the inorganic serum phosphorus serves as an index to pituitary growth hormone production and/or effect The phosphorus level is high prior to puberty falls to the normal level during puberty and can be brought to normal in acromegalics and giants by the administration of anabolic steroids This then may be interpreted as indicating that with the onset of puberty the production of pituitary growth hormone is inhibited to a significant degree thereafter The fact that no significant elevation of serum phosphorus oc curs in the female after the menopause might suggest that its production is not resumed in significant amounts at this time One might add that the progression of senescence is even more suggestive of such an interpretation Dr Hamilton tells us that in young adult males following castration the serum phosphorus level promptly increases [1] This suggests a post castration increase in pituitary growth hormone production in young subjects

Regarding question two it would seem most unlikely to us that multiple

hormones must be co administered in order to produce any significant anabolic effect On the other hand the evidence for multiple pituitary dysfunctions in patients with acromegaly is well known

As in question 2 it would seem to us quite improbable that the answer to question 3 is in the affirmative However it still remains to be finally excluded

Regarding question 4 it would seem to us that there is considerably more evidence against, than for the thesis that pituitary growth hormone and the non ACTH pituitary diabetogenic factor are identical In Figure 8 is presented diagrammatically a concept based upon duality rather than unity Growth requires energy If one conceives of the diabetogenic factor as having as its normal function the mobilization of fat and fat derivatives for this purpose with resultant formation of small molecular fragments *which*

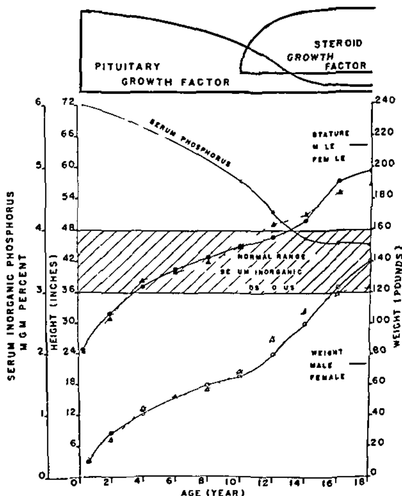


Fig 7 Hormonal regulation of growth—1954 concept

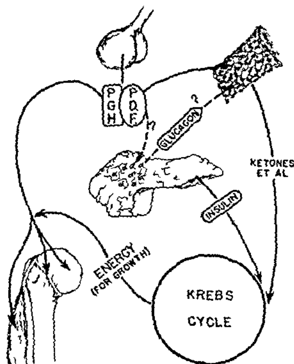


FIG 8 It is postulated that the pituitary diabetogenic factor is concerned with energy mobilization for support of growth

require insulin for their total oxidation the normal function of this factor would be apparent and its ability to produce the diabetic state in an individual with beta cells of less than normal vigor would be explained

It has been suggested that the pituitary diabetogenic factor (P D F) may act via the α cells of the pancreas with resultant increase in glucagon production Recently we have administered glucagon chronically to non diabetic and diabetic subjects during complete fast No significant effect on blood sugar or ketones was observed in the non diabetics (as compared to a control fast in the same subject) Extreme accentuation of hyperketonemia occurred in a diabetic patient (Figure 9) There was only a moderate increase in hyperglycemia as compared to the control fast No insulin was administered to the diabetic during either the control fast or the glucagon fast If glucagon were the energy mobilizer in response to the stimulation by the pituitary diabetogenic factor such an effect would be expected in an insulin dependent mechanism

The strongest argument against the concept of the action of P D F via the α cells is the production of increased hyperglycemia in *pancreatecto*

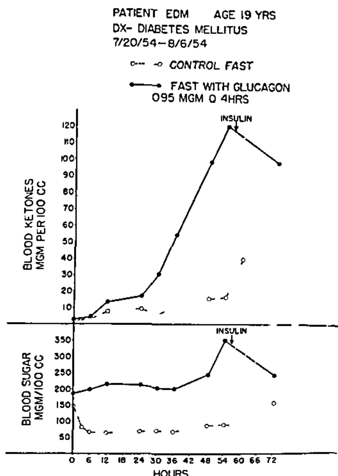


FIG 9 Production of keto acidosis with glucagon in a fasting diabetic without antecedent or proportional increase in blood sugar

mized animals following the administration of purified pituitary growth hormone '

Recently we have carried out hypophysectomy in a selected group of juvenile diabetics with progressive vascular disease All of these patients *had high insulin requirements prior to surgery* and all have had rapid and major decreases in insulin requirements following hypophysectomy

Adrenalectomy carried out in similar patients by Hamwi[3] and Conn[4] results in little or no decrease in insulin requirement Both the hypophysectomized and the adrenalectomized patients received maintenance amounts of corticoids following surgery

These observations would suggest strongly that in spontaneous and severe juvenile diabetes the pituitary factor responsible for insulin resistance is not ACTH but the pituitary diabetogenic factor associated with growth If juvenile diabetes, then is habitually associated with hyper production of

this factor and if the growth factor and diabetogenic factor are identical one should expect a high incidence of acromegaly or gigantism in juvenile diabetics This is not the case and it is suggestive evidence certainly that the growth factor and the diabetogenic factor are *not* one and the same

In conclusion it would seem to us that despite the vicissitudes which have beset everyone who has worked in this field there is good reason to pursue such work until a suitable set of answers is found The problem can only be solved by objective collaborative activity between protein chemists physiologists and clinical physiologists

References

1 Hamilton Joseph B Personal communication
2 Lukens F D W *Can Med Assoc J* 65 334 (1951)
3 Hamwi George Personal communication
4 Conn Jerome Personal communication

29

Metabolic Studies of the Action of Growth Hormone (Somatotropin) in Man*

Ephraim Shorr Anne C Carter, Richmond W Smith Jr, Byrl J Kennedy † Richard J Havel Thomas N Roberts Lawrence L Sonkin and Ernest T Livingstone With the technical assistance of Vincent Toscani Estelle Stevens Viola Davis Mary Dittbrenner and Mary P Martin

Russell Sage Institute of Pathology The Department of Medicine Cornell University Medical College and The New York Hospital New York

Although the growth promoting properties of crude anterior pituitary extracts had been demonstrated by Evans and Long as early as 1921 [1] and Li Evans and Simpson had succeeded in 1944 in isolating a specific protein with these properties from the anterior pituitary it was not until 1948 when Wilhelmi Fishman and Russell[3] developed a procedure for crystallizing growth hormone in good yield that a wider exploration of its biological properties in animals and man was possible In the intervening years a rich literature has accumulated on the effects of this hormone in animals not only on growth but on a wide variety of metabolic processes as well Evidence for its action in man is on the other hand meager and inconclusive The available data on human subjects are in conflict as to whether growth hormone can acheive protein or other anabolic effects or influence carbohydrate metabolism [4, 6, 7, 8, 9, 10, 11, 1, 13]

The purpose of this report is to present the data obtained during the past six years on six human subjects who received crystalline growth hormone (somatotropin) prepared by the Armour Laboratories according to the

* Aided by grants from the Playtex Park Research Institute and The New York Foundation Inc We are indebted to the Armour Laboratories Chicago for the somatotropin used in these studies

† Damon Runyon Fellow

method of Wilhelmi Fishman and Russell The subjects were studied in the Research Metabolism Ward of the Russell Sage Institute of Pathology and were under strict dietary control throughout the period of study

Plan of Study

The three major problems involved in the detailed design of this study were (1) the selection of subjects who might be most appropriate for the study of the action of somatotropin (STH) (2) the choice of metabolic indices which were most likely to be responsive to the hormone and (3) the provision of a dietary intake whose composition would be favorable for the demonstration of its anabolic properties

The ideal subject would seem to be one who is normal in all respects except for a deficiency in growth and without a genetic factor limiting the capacity of the tissues to respond to a growth stimulus However in addition to the difficulty of securing reliable criteria for making such judg ments in the case of man subjects are not always available who are tailored to one s needs The six subjects four males and two females had one feature in common short stature as compared to the mean for their age In Table 1 some of the data are given of the growth and endocrine status of these subjects a more complete account of each subject is given below Two of the six had diminished thyroid function a circumstance which provided an opportunity to determine whether the action of STH might be dependent upon the presence of the thyroid hormone Four of the subjects were puberal in two gonadal function was absent Four of the subjects showed some degree of insulin sensitivity none had any demon strable disturbance in electrolyte metabolism One had platybasia another a mild osteochondrodystrophy one a family history of short stature with two cousins who were dwarfed and one had a craniopharyngioma which had previously been aspirated for the relief of increased intracranial pressure

The metabolic indices were selected for measurement on the basis of the action of STH in animals in augmenting the storage of protein phosphorus and calcium in elevating the serum inorganic phosphorus and alkaline phos phatase and in inducing contra insulin effects such as glycosuria impaired glucose tolerance and increased insulin resistance In view of the possible contamination of the STH preparations with other hypophyseal hormones measurements were also made throughout the study of thyroid adrenal and gonadal function

The following data were obtained daily height weight fluid intake and urine output continuous metabolic balance studies for nitrogen calcium phosphorus sodium and potassium basal metabolic rates and in the female subjects vaginal smears every other day *Blood Studies* serum inorganic phosphorus alkaline phosphatase calcium total protein sodium potassium chlorides cholesterol protein bound iodine carbon dioxide combining power and urea nitrogen eosinophil counts and glucose and

29

Metabolic Studies of the Action of Growth Hormone (Somatotropin) in Man*

Ephraim Shorr Anne C Carter, Richmond W Smith Jr Byrl J Kennedy † Richard J Havel Thomas N Roberts Lawrence L Sonkin and Ernest T Livingstone With the technical assistance of Vincent Toscani Estelle Stevens Viola Davis Mary Dittbrenner and Mary P Martin

Russell Sage Institute of Pathology The Department of Medicine Cornell University Medical College and The New York Hospital New York

Although the growth promoting properties of crude anterior pituitary extracts had been demonstrated by Evans and Long as early as 1921[1] and Li Evans and Simpson had succeeded in 1944 in isolating a specific protein with these properties from the anterior pituitary it was not until 1948 when Wilhelmi Fishman and Russell[3] developed a procedure for crystallizing growth hormone in good yield that a wider exploration of its biological properties in animals and man was possible In the intervening years a rich literature has accumulated on the effects of this hormone in animals not only on growth but on a wide variety of metabolic processes as well Evidence for its action in man is on the other hand meager and inconclusive The available data on human subjects are in conflict as to whether growth hormone can acheive protein or other anabolic effects or influence carbohydrate metabolism[4 5 6 7 8 9 10 11 1 13]

The purpose of this report is to present the data obtained during the past six years on six human subjects who received crystalline growth hormone (somatotropin) prepared by the Armour Laboratories according to the

* Aided by grants from the Playtex Park Research Institute and The New York Foundation Inc We are indebted to the Armour Laboratories Chicago for the somatotropin used in these studies

† Damon Runyon Fellow

method of Dunger[23] Basal metabolic rates were done with a Sanborn Waterless Metabolism Tester and calculated from the Aub DuBois stand ards Vaginal smears were stained by the method of Shorr [8]

The Armour somatotropin which is prepared from beef pituitaries was diluted without shaking with from 1 5 to 3 ml of either sterile distilled water unbuffered physiological saline or with some lots physiological saline buffered to pH 11 0 in order to achieve on mixing a pH of 9 3 at which this STH is soluble Approximately 10% of the total nitrogen per vial of lot G411 did not go into solution and it was necessary to centrifuge each vial after diluting Only the supernatant was injected Lots H1802 and H1902 both representing aliquots of the same preparation were re ceived in the frozen state from the Armour Laboratories A portion was kept frozen and thawed just before use the remainder was lyophilized in our laboratory The solutions of lyophilized STH were always made up im mediately prior to their intramuscular injection half the daily dose being given in the morning the remainder in the evening The pork growth hor mone of Raben and Westermeyer which was kindly furnished by these in vestigators was prepared for injection in the following manner to a 3 day supply sterile distilled water was added equal to 2 ml per injection The mixture was stirred with a sterile glass rod while concentrated HCl was added dropwise from a capillary tube until the hormone went into solution A drop was tested with bromphenol blue paper to ensure that the pH lay between 3 0 and 3 5 This solution was then kept in the refrigerator and used up over a three day period

Results

The first subject L F (f) N Y H 479 918 was 14 years 7 months old at the time the study was begun in 1948 She had been small since infancy and linear growth had always been appreciably below normal standards with however no delay in walking talking or in mental development Previous therapy with desiccated thyroid (90 mg per day) had not altered her growth rate Thyroid therapy had been discontinued several years prior to this study Breast development began 12 months and pubic hair had appeared 6 months before her admission but she had not as yet menstru ated Her parents were short her father measuring 5 feet 6 and ½ inches and her mother 5 feet 3 inches but 3 younger siblings were of normal height

Physical Examination on Admission Weight 29 3 kg height 136 6 cm sitting height 69 2 cm arm span 136 5 cm The bodily contour was pre puberal and symmetrical and the nutritional status good (Fig 1) There were a few hairs on the labia majora but no axillary hair growth The outer half of the eyebrows were thin The breasts were small and conical and no glandular tissue was palpable Blood pressure was 90/58 There were soft systolic murmurs at the apex and pulmonic regions regarded as functional

insulin tolerance tests *Urinary Analyses* daily nitrogen, glucose, acetone bodies creatine and creatinine weekly output pooled for phosphorus calcium sodium potassium sulfur, citric acid and 17 ketosteroid excretion biweekly creatine tolerance tests on creatine creatinine free diets and 11 oxysteroids

The composition of the diets with respect to nitrogen phosphorus, calcium sodium and potassium was such that all the subjects were in positive balance for each component during the control period preliminary to the administration of STH Adequate calories were provided to maintain weight The diets were supplemented with vitamins to the extent of half the currently accepted daily requirements The subjects not receiving desiccated thyroid were given 13 mg iodine daily in the form of syrup of hydriodic acid

Methods

The methods used in this study were those routinely employed on the Metabolism Ward of the Russell Sage Institute of Pathology [14 1 16]

The metabolic balance studies were carried out for seven day periods They were not begun until the subjects were well adjusted to their diets and had been receiving them for at least 7 days Each subject received in rotation three different daily menus which were approximately isocaloric and contained nearly equal amounts of nitrogen phosphorus calcium sodium and potassium One creatine creatinine free diet was provided for each subject and used during biweekly creatine tolerance tests The caloric and mineral contents of the diets were calculated from the values given in Sherman [17] sodium and potassium values were obtained from the Mead Johnson tables [18] From time to time during the experiments the diets were analyzed by the in toto method the results were in sufficiently good agreement with the calculated values to validate the use of the latter

Urine specimens were preserved by refrigeration the 7 day pooled specimens were made up of aliquots of the 24 hour collections and acidified with 10 ml of concentrated HCl The 11 oxysteroids were analyzed by a modification of the method of Daughaday et al [19] urinary gonadotropins by the method of Gorbman [0] or Smith et al [21]

All blood samples were drawn in the fasting state The serum alkaline phosphatase was analyzed by the phenolphthalein monophosphate method of Gutman [2] chlorides by the method of Schales and Schales [3] carbon dioxide combining power by the method of Van Slyke and Cullen [24] cholesterol by the method of Schoenheimer and Sperry [5] and protein bound iodine by the method of Barker et al [6] Blood sugars were analyzed by the method of Nelson[27] using finger tip blood Oral glucose tolerance tests were employed the subjects receiving 1 75 g glucose per kg For the intravenous insulin tolerance tests 0 03 to 0 1 unit of insulin per kg were given

Eosinophil counts were done on chilled heparinized venous blood by the

Thyroid status B M R minus 20% serum cholesterol 352 mg % serum protein bound iodine 5 8 gamma % I^{131} excretion in 48 hours 74 4% *Adrenal status* Serum sodium 136 5 potassium 5 0 chlorides 108 and CO combining power 28 mEq / l eosinophils 127 per mm^3 with an 80% fall following a 4 hour intramuscular ACTH test normal response to 4 day salt withdrawal test urinary 17 ketosteroids 2 47 mg and 11 oxysteroids 0 92 mg per 24 hours mild insulin hypersensitivity (see Fig 3) *Gonadal func*

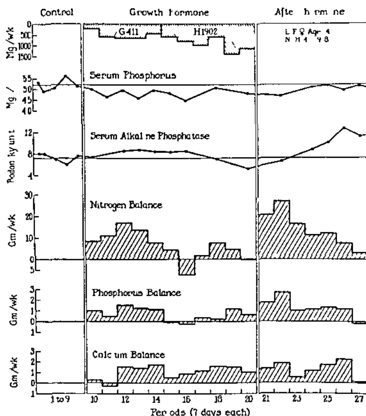


FIG 2 L F (f) 14 yrs 7 mos On constant daily intake of 2 102 calories 89 4 g protein (14 3 g nitrogen) 1 619 g phosphorus and 1 500 g calcium During the 9 week control period which served as the baseline for STH studies the subject was in positive balance with respect to nitrogen of 9 01 g per week to phosphorus of 1 078 g per week and to calcium of 1 281 g per week In this as well as subsequent Figures the metabolic data obtained during or after the administration of STH are charted as positive or negative deviations from the control values The *total* storage of N P and Ca during any period may be obtained from the algebraic sum of the positive values during the control period and the deviations observed during or after STH administration The serum inorganic phosphorus and alkaline phosphatase values represent individual de terminations the straight horizontal line representing the average of the control periods

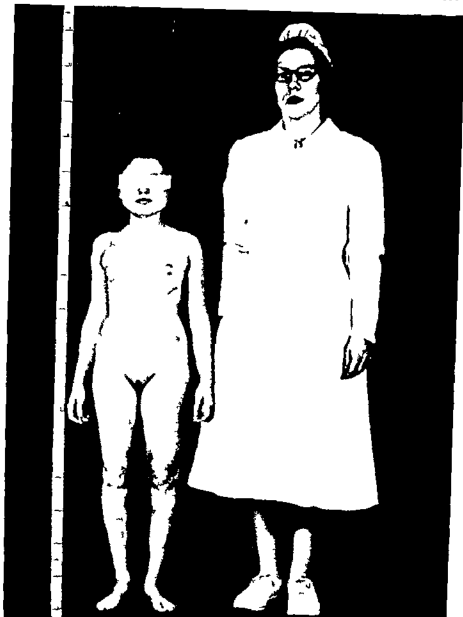


FIG 1 NYH 479 918 Pre treatment status Age 14 yrs 7 mos Ht 136 6 cm Wt 29 3 kg

Laboratory Data on Admission The urine was negative for albumin and sugar *Renal function* maximal concentration 1 018 dilution to 1 000 PSP 80% in 2 hours, urea clearance 60% *Hemogram* Hb 13 5 g R B C s 4 4 million per mm³ hematocrit 41 W B C s 8 700 with a normal differential count *EKG* normal, with sinus irregularity *Visual fields* normal *X ray of skull* normal *Bone age* 10 years 9 months (Todd s standards)

Thyroid status B M R minus 20% serum cholesterol 352 mg % serum protein bound iodine 5 8 gamma % I^{131} excretion in 48 hours 74 4% *Adrenal status* Serum sodium 136 5 potassium 5 0 chlorides 109 and CO combining power 28 mEq l eosinophils 127 per mm³ with an 80% fall following a 4 hour intramuscular ACTH test normal response to 4 day salt withdrawal test urinary 17 ketosteroids 2 47 mg and 11-oxysteroids 0 92 mg per 24 hours mild insulin hypersensitivity (see Fig 3) *Gonadal func*

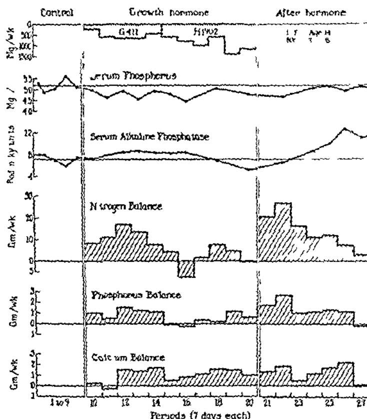


FIG 2 L F (f) 14 yrs 7 mos On constant daily intake of 2 102 calories 89 4 g protein (14 3 g nitrogen) 1 619 g phosphorus and 1 500 g calcium During the 9 week control period which served as the baseline for STH studies the subject was in positive balance with respect to nitrogen of 9 01 g per week to phosphorus of 1 078 g per week and to calcium of 1 281 g per week In this as well as subsequent Figures the metabolic data obtained during or after the administration of STH are charted as positive or negative deviations from the control values The *total* storage of N P and Ca during any period may be obtained from the algebraic sum of the positive values during the control period and the deviations observed during or after STH administration The serum inorganic phosphorus and alkaline phosphatase values represent individual de terminations the straight horizontal line representing the average of the control periods

tion vaginal smears, atrophic urinary gonadotropins less than 5 mouse units per 24 hours *Blood* serum calcium 11 0 mg % inorganic phosphorus 5 31 mg %, alkaline phosphatase 7 9 Bodansky units total proteins 6 91 g % Results of sugar tolerance and insulin tolerance tests are given elsewhere (see Fig 3)

The data relating to nitrogen, calcium and phosphorus balances and the serum inorganic phosphorus and alkaline phosphatase levels are presented in graphic form in Figure 2 Throughout the study she was on a constant dietary intake of 2 102 calories made up of 89 4 g protein, 103 9 g fat 202 4 g carbohydrate and containing 1 500 g calcium and 1 619 g phos phorus per day *This diet was adequate to maintain average positive bal* ances of nitrogen 9 01 g calcium 1 281 g and phosphorus 1 078 g per week during the 9 week control period which provided the baseline data for the evaluation of the effects of STH Seven day balance periods were em ployed throughout and are plotted to give the *total* positive or negative values for each seven day period in relation to the control level for each dietary component Increases above the control values are plotted above, and decreases below the baseline The serum inorganic phosphorus and alkaline phosphatase values represent individual determinations the straight horizontal line representing the average of the control periods Two lots of Armour STH were administered over a period of 11 weeks The total dose administered per 7 day period is given at the top of the chart The daily dose averaged 112 mg with a maximum of 300 mg per day Before the hormone was given the patient was skin tested with Lot G411 and gave a negative response However, both lots of hormone used with this patient induced considerable local tenderness and discomfort which was not relieved or prevented by the addition of procaine to the STH at the time of injection On one occasion, the injection of Lot H1902 was followed immediately by abdominal cramps cough flush and a temperature rise to 38 2° C From the bleeding apparent at the injection site this was presumed to have re sulted from the hormone having entered a vein This episode was rapidly terminated by an injection of epinephrine Thereafter, the subject continued to tolerate the hormone well except for the local tenderness mentioned above

The administration of STH was followed by a prompt anabolic effect on nitrogen metabolism which was maximal in the third week during which 15 0 g of extra nitrogen were stored This was followed by a decline and then a fall below the baseline with the subsequent return of an anabolic effect of smaller magnitude A similar increase in phosphorus storage occurred with fluctuations which paralleled those observed for nitrogen An increased retention of calcium was noted by the third week and persisted with minor fluctuations throughout the period of hormone administration

After STH was discontinued because of the development of frank gly cosuria there occurred a sharp augmentation of storage of nitrogen and

phosphorus along with the persistent increased retention of calcium A return to the initial control values did not take place until the seventh week A similar phenomenon after stopping STH was encountered in one other patient in this study subject R V

During the period of administration serum inorganic phosphorus values fell below the baseline values by an average of 0 3 mg per cent and alkaline phosphatase rose slightly on an average of 0 6 B U After the hormone was discontinued there was a slow steady increase in the alkaline phosphatase which reached a maximum of 12 8 B U during the sixth week

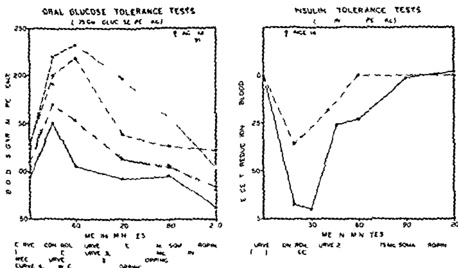


FIG 3

Oral glucose tolerance curves which were obtained at intervals throughout the study are given in Figure 3 They show the progressive development of a diabetic glucose tolerance curve most marked at the end of the eleventh week after the subject had received a total of 9 375 mg of STH and showed a frank glycosuria of 1 75 g per 24 hours Repetition of the glucose tolerance curves 6 and 31 weeks after discontinuing STH showed a slow regression towards control values The glycosuria on the other hand disappeared promptly on stopping therapy Insulin tolerance curves on the same subject are also shown in Figure 3 The curve obtained during the control period showed a moderate insulin hypersensitivity Curve 2 obtained after eleven weeks of STH indicated the development of insulin resistance

In view of the absence of detectable amounts of ACTH in the STH preparations used in this study the changes in urinary 17 ketosteroids and 11 oxysteroids of this subject during STH administration were unexpected During the control periods the 17 ketosteroids averaged 2 49 mg per 24 hours and one determination of 11-oxysteroids gave a value of 0 92 mg per

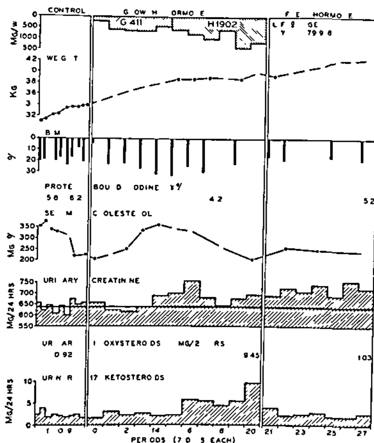


Fig 4

24 hours During the seventh week of STH the 17 ketosteroid value rose to 5 95 mg and remained at approximately that level through the tenth week In the eleventh or last week of STH the level had risen to 9 65 mg and the 11 oxysteroids to 9 45 mg per 24 hrs After discontinuing STH there was an abrupt fall in 17 ketosteroids to the control values where they remained throughout the remainder of the study (Fig 4) The 11 oxysteroid value at the end of the study period had also fallen to the control levels There were no changes in the absolute eosinophil counts during the period of study In no other subject receiving STH was there any significant changes in urinary 17 keto- or 11-oxysteroids

Sodium balances were positive throughout the study the average weekly retention during the control periods was 4 032 g which increased to 7 532 g during STH and rose to 9 443 g in the post periods This sodium retention was not associated with any change in fluid balance or serum electrolytes Potassium balances were positive throughout the period of study but no correlation with nitrogen balances was apparent

Because of contamination of the STH preparations with small amounts

of thyrotropin (TSH) thyroid function was carefully studied and she was given an iodine supplement of 13 mg daily throughout the study The basal metabolic rate averaged minus 18% during the control period minus 24% during STH administration and minus 18% in the post treatment period The serum cholesterol values which are given in Fig 4 showed a curious slow fluctuation between extremes of 374 and 200 mg % unrelated to medication The serum protein bound iodine remained within the normal range throughout From these findings it is evident that thyroid function was unaffected by the STH despite its TSH content Assays of the STH used in this study showed no contamination with gonadotropic hormones and the vaginal smears remained atrophic throughout the study The urinary citric acid remained relatively constant

The changes in urinary creatinine were regarded as significant (Fig 4) The average daily urinary creatinine was 0 639 g during the control periods 0 671 g during the 11 weeks of STH and 0 720 g for the 7 post treatment periods the last value representing an increase of 13% over the control level The extra nitrogen stored during this same period of time was equivalent to 4 6 kg of protoplasm or approximately 13% of the weight of the patient at the time STH was given There were no significant changes in the daily creatinuria or creatine tolerance tests

This subject received 67 calories per kg as compared with 51–56 calories per kg *taken by the other subjects in this study Her weight gain averaged* 0 29 kg per week during the control period and 0 57 kg per week during

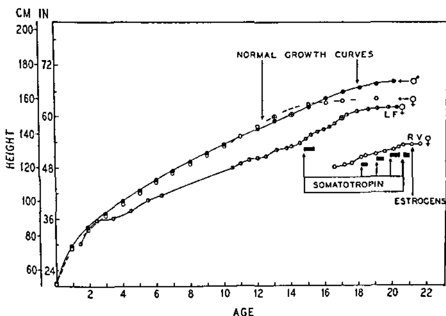


FIG 5 Growth curves of the female subjects L F and R V

STH therapy Her height increased 0 5 cm during the 9 week control period 1 5 cm during the 11 periods of hormone therapy and 1 1 cm in the 7 post therapy weeks In the ensuing 3 months she grew an additional 0 7 cm Her subsequent history is as follows menstruation began at the age of 17 and at the age of 19, with the epiphyses still open and a bone age of 15 years 3 months she had attained a height of 154 9 cm an increase of 18 cm in the four years since receiving STH (Fig 5)

The second subject R V (f) N Y H 586 955 was admitted in 1950 and was studied for four years during which she received 10 lots of Armour STH and one prepared by Raben and Westermeyer At the time of admission she was 17 years 10 months old Since the age of 2 or 3 her rate of growth and weight gain had been below normal standards Deciduous teeth were not lost until 12 years however there had been no delay in walking talking or mental development Previous therapy elsewhere with desiccated thyroid (60 mg per day) methyl testosterone (5 mg per day) injections of estrogens chorionic gonadotropic hormone and growth hormone had not altered her growth rate Thyroid and methyl testosterone therapy were discontinued two weeks prior to her first admission She had no axillary or pubic hair and had not menstruated Her mother was 5 feet 1 inch tall one paternal uncle 5 feet all other relatives were between 5 feet 4 inches and 5 feet 11 inches in height

Physical Examination on Admission Weight 25 7 kg height 124 8 cm sitting height 64 0 cm arm span 121 8 cm The bodily contour was infantile and symmetrical and the nutritional status good (Fig 6) There were a few fine hairs on the labia majora but no axillary hair The skin was dry and rough The breasts were questionably enlarged probably due to fat The genitalia were infantile Blood pressure was 95/65 There were soft systolic murmurs at the apex and pulmonic regions regarded as functional There was a bilateral nystagmus Reflexes were normal in 1950 although by 1952 reflexes were absent in the upper extremities and abdomen and hyperactive in the lower extremities with equivocal plantar responses Vibration sense was absent below the upper extremities

Laboratory Data on Admission The urine was negative for albumin and sugar *Renal function* maximal concentration 1 027 dilution to 1 002 PSP 85% in 2 hours urea clearance 71 8% *Hemogram* Hb 11 2 g R B C s 4 2 million per mm^3 hematocrit 38 W B C s 7 500 with a normal differential count *E K G* normal with sinus irregularity *E E G* normal for a child of 5 to 7 years *Visual fields* normal *X ray of skull* multiple unerupted teeth otherwise normal Special films in 1952 platybasia and basilar impression *Bone age* 11 years 3 months (Todd s standards) *Thyroid status* B M R minus 13% serum cholesterol 248 mg % serum protein bound iodine 4 0 gamma % I^{131} neck uptake 10 7% and excretion 83 2% in 48 hours *Adrenal Status* serum sodium 135 2 potassium 5 0 chlorides 109 and CO combining power 23 mEq /l eosinophils 322 per

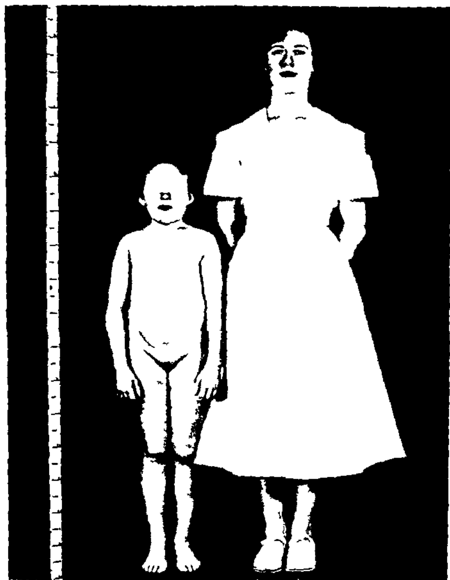


FIG 6 NYH 586 955 Pre treatment status Age 17 yrs 10 mos Ht 124 8 cm Wt 25 7 kg

mm³ with a 56% fall following a 4 hour intramuscular ACTH test normal response to 4 day salt withdrawal test urinary 17 ketosteroids 2 92 mg and 11-oxysteroids 0 61 mg per 24 hours mild insulin hypersensitivity (Fig 10) *Gonadal function* vaginal smears atrophic urinary gonadotropins less than 5 mouse units per 24 hours *Blood* serum calcium 10 4 mg % inorganic phosphorus 4 17 mg % alkaline phosphatase 5 2 B U total

STH therapy Her height increased 0 5 cm during the 9 week control period 1 5 cm during the 11 periods of hormone therapy and 1 1 cm in the 7 post therapy weeks In the ensuing 3 months she grew an additional 0 7 cm Her subsequent history is as follows menstruation began at the age of 17 and at the age of 19 with the epiphyses still open and a bone age of 15 years 3 months she had attained a height of 154 9 cm an increase of 18 cm in the four years since receiving STH (Fig 5)

The second subject R V (f) N Y H 586 955 was admitted in 1950 and was studied for four years during which she received 10 lots of Armour STH and one prepared by Raben and Westermeyer At the time of admission she was 17 years 10 months old Since the age of 2 or 3 her rate of growth and weight gain had been below normal standards Deciduous teeth were not lost until 12 years however there had been no delay in walking talking or mental development Previous therapy elsewhere with desiccated thyroid (60 mg per day) methyl testosterone (5 mg per day) injections of estrogens chorionic gonadotropic hormone and growth hormone had not altered her growth rate Thyroid and methyl testosterone therapy were discontinued two weeks prior to her first admission She had no axillary or pubic hair and had not menstruated Her mother was 5 feet 1 inch tall one paternal uncle 5 feet all other relatives were between 5 feet 4 inches and 5 feet 11 inches in height

Physical Examination on Admission Weight 25 7 kg height 124 8 cm sitting height 64 0 cm arm span 121 8 cm The bodily contour was infantile and symmetrical and the nutritional status good (Fig 6) There were a few fine hairs on the labia majora but no axillary hair The skin was dry and rough The breasts were questionably enlarged probably due to fat The genitalia were infantile Blood pressure was 95/65 There were soft systolic murmurs at the apex and pulmonic regions regarded as functional There was a bilateral nystagmus Reflexes were normal in 1950 although by 1952 reflexes were absent in the upper extremities and abdomen and hyperactive in the lower extremities with equivocal plantar responses Vibration sense was absent below the upper extremities

Laboratory Data on Admission The urine was negative for albumin and sugar *Renal function* maximal concentration 1 027 dilution to 1 002 PSP 85% in 2 hours urea clearance 71 8% *Hemogram* Hb 11 2 g R B C s 4 2 million per mm^3 hematocrit 38 W B C s 7 500 with a normal differential count *E K G* normal with sinus irregularity *E E G* normal for a child of 5 to 7 years *Visual fields* normal *X ray of skull* multiple unerupted teeth otherwise normal Special films in 1952 platybasia and basilar impression *Bone age* 11 years 3 months (Todd s standards) *Thyroid status* B M R minus 13% serum cholesterol 248 mg % serum protein bound iodine 4 0 gamma % I^{131} neck uptake 10 7% and excretion 83 2% in 48 hours *Adrenal Status* serum sodium 135 2 potassium 5 0 chlorides 109 and CO combining power 23 mEq /l eosinophils 322 per

The results of the first year of study are given in Figure 7 The interpretation of the data is complicated by the appearance of signs and symptoms of thyroid insufficiency during the eighth week of STH administration at which time serum cholesterol had risen to 336 mg % and the protein bound iodine fallen to 2 gamma % After a 6 week control period she was given two lots of STH Lot J21609R was administered for 10 weeks the daily dose averaged 112 mg with a maximum of 250 mg per day During this STH period there were no changes in the nitrogen or phosphorus balances but there did occur a moderate average increase in calcium retention of 0 791 g per week greater than during the control period Another lot of STH was given for 2 weeks before and continued for 4 weeks after thyroid therapy was instituted A daily maintenance dose of 112 mg desiccated thyroid was given throughout the remainder of the study Soon after thyroid therapy was initiated she went into significant negative nitrogen and phosphorus balance and eventually into negative calcium balance as compared with her control values There was an average increase in creatinuria of 152 mg per day The serum inorganic phosphorus rose on the average of 0 6 mg % during thyroid therapy

Seven weeks after STH was discontinued and nitrogen phosphorus and calcium balances had returned to baseline values the first STH preparation was readministered for 3 weeks this time with a distinct enhancement of the storage of all three dietary components During this entire period of study there were no significant changes in carbohydrate metabolism

The data of the second day of study during which time the patient was maintained on desiccated thyroid 112 mg per day are given in Figure 8 After a 7 week control period two lots of STH were given in succession over an 11 week period with an average dose of 217 mg and a maximal daily dose of 280 mg Lot J21609R again caused an extra storage of nitrogen of the same order of magnitude as at the end of the previous study (Fig 7) Lot K43806R produced an extra storage of nitrogen although to a lesser degree The anabolic effects wore off towards the end of therapy and there was no post treatment retention There was no appreciable extra retention of phosphorus or calcium indeed the storage of both fell below the control values toward the end of therapy The serum inorganic phosphorus values rose slightly on an average of 0 4 mg % the serum alkaline phosphatase remained stable There were no changes in either glucose or insulin tolerance tests The absolute eosinophil count rose from an average control level of 166 to 601 per mm³ during STH therapy falling to control levels during the post STH periods without any alterations of other indices of adrenal function

After an 8 week interval she received 1 700 mg of the Raben and Westermeyer STH in 16 days During the third week the nitrogen phosphorus and calcium balances became strongly negative to return promptly to the baseline values on cessation of treatment She was then given a third

proteins 7 8 g % Results of sugar and insulin tolerance tests are given else where (Fig 10)

During the 4 year study period she was on an average constant dietary intake of 1,496 calories made up of 66 1 g protein, 75 1 g fat 139 g carbohydrate and containing 1 500 g calcium and 1 497 g phosphorus per day This diet was adequate to maintain her in positive nitrogen calcium and phosphorus balance during the control periods prior to the adminis tration of STH

Before each lot of STH was given the patient was skin tested and gave negative responses The subject had a chill with temperature rise to 40 6° C 4 hours after an injection of the Raben and Westermeyer preparation having received a total dose of 1 700 mg in the preceding 16 days The fever subsided in 18 hours however the skin test had now become positive to this preparation Lot K491132 caused a chill and temperature rise on one occasiton while Lot R527093 produced daily temperature elevations to between 38 and 39° C Some lots of STH produced considerable local tenderness and discomfort at the site of injection

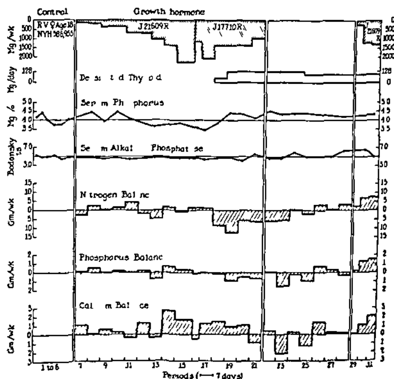


Fig 7 R V (f) 17 yrs 10 mos On constant daily intake of 1 414 calories 63 9 g protein (10 2 g nitrogen) 1 507 g phosphorus and 1 499 g calcium During the 6 week control period the subject was in positive balance with respect to nitrogen of 12 46 phosphorus of 0 637 and calcium of 0 931 g per week

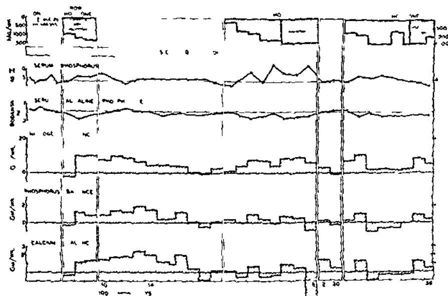


FIG 9 R V (f) 19 yrs 10 mos On constant daily intake of 1 528 calories 65 8 g protein (10 5 g nitrogen) 1 507 g phosphorus and 1 500 g calcium During the 6 week control period the subject was in positive balance with respect to nitrogen of 14 84 phosphorus of 2 884 and calcium of 3 010 g per week

During this course of STH the subject showed for the first time changes in glucose and insulin tolerance curves (Fig 10) The glucose tolerance curve returned to control levels after 16 days and the insulin tolerance 35 days after stopping STH There was no glycosuria and no changes in fasting blood sugar levels or in eosinophil counts

The next two lots of STH R491276 and R527026B were given in succession over an 8 week period again with an unequivocal anabolic effect on nitrogen storage, but with only a slight enhancement of phosphorus retention and no effect on calcium storage The serum inorganic phosphorus was elevated on the average of 0 4 mg % and the alkaline phosphatase lowered 0 9 B U both changes acquiring additional significance by their prompt return to control levels after STH was stopped The eosinophils increased from an average control value of 197 to 334 mm³ Both lots of STH produced a tachycardia without fever an elevation of 13 per cent in the basal metabolic rate above the average control level without any changes in serum cholesterol or protein bound iodine During the final 8 week study period Lot R527093 and R527093C produced an enhanced storage of nitrogen although of lesser magnitude but no significant alterations in phosphorus or calcium retention There was a slight transient elevation in serum inorganic phosphorus of 0 16 mg % and a sustained lowering of the alkaline phosphatase of 0 4 B U The eosinophils rose to 509 compared to a

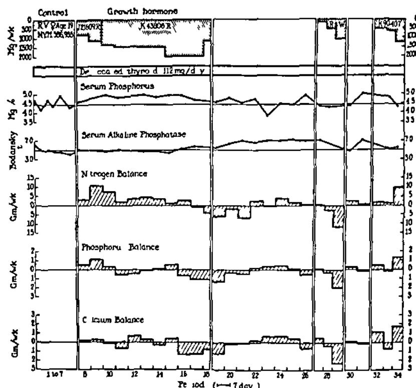


FIG 8 R V (f) 18 yrs 10 mos On constant daily intake of 1 429 calories 64 6 g protein (10 3 g nitrogen) 1 460 g phosphorus and 1 492 g calcium During the 7 week control period the subject was in positive balance with respect to nitrogen of 13 51 phosphorus of 1 827 and calcium of 1 967 g per week

Armour STH for 3 weeks in a total amount of 2 350 mg and went into positive balance with respect to all three dietary components during the third week There again was a rise in the absolute eosinophil count No post treatment data were obtained because of the summer closing of the *Metabolism Ward*

The third year of study was resumed in the fall of 1952 and continued for a period of 38 weeks (Fig 9) After a 6 week control period she was given a fifth lot of Armour STH for 20 days in a total dosage of 3 450 mg with an average daily dose of 173 mg There were striking increases in the storage of nitrogen phosphorus and calcium during the last two periods of hormone administration The maximal retentions above the control were nitrogen 11 83 phosphorus 1 407 and calcium 1 421 g per week Following the discontinuation of STH she continued for 8 weeks to store these dietary constituents in excess of the control values

There was a slight rise in the serum inorganic phosphorus averaging 0 2 mg % and a fall in alkaline phosphatase on the average of 1 2 B U both returning rapidly to control levels after STH was discontinued

of 50, 100, 200 mg over 17 weeks following an 8 week control period The extra retention of nitrogen was intermittent during the first 13 periods however it was well sustained at an average value of 4 8 g per period during the last 4 weeks No significant anabolic effects on phosphorus and calcium were observed The serum inorganic phosphorus was unchanged whereas the alkaline phosphatase was elevated on an average of 1 0 B U The eosinophils rose from an average control level of 291 to 990 mm³ There were no alterations in carbohydrate metabolism

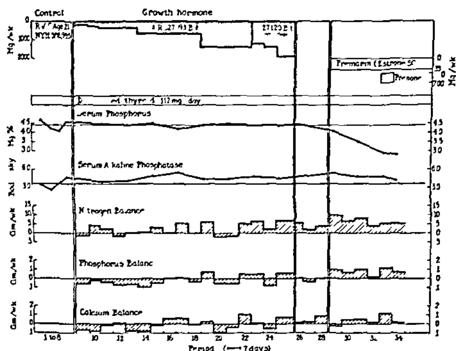
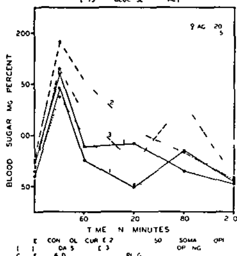


Fig 12 R V (f) 20 yrs 10 mos On constant daily intake of 1 612 calories 70 2 g protein (11 2 g nitrogen) 1 517 g phosphorus and 1 512 g calcium During the 8 week control period the subject was in positive balance with respect to nitrogen of 15 26 phosphorus of 2 765 and calcium of 3 325 g per week

During the 4 years of intermittent STH administration to the subject there were no significant changes in any of the other indices studied such as creatinine 17 ketosteroids 11 oxysteroids serum electrolytes and sodium balances She gained a total of 3 56 kg in weight and grew 6 9 cm in height (Fig 5) Her bone age increased from 11 years 3 months at the chronological age of 17 years 10 months to 12 years and 9 months at a chronological age of 21 years 5 months

During the last 6 weeks of the study the patient received conjugated estrone sulfate (Premarin®) 5 mg daily equivalent to 3 200 R U on the



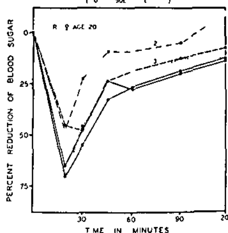
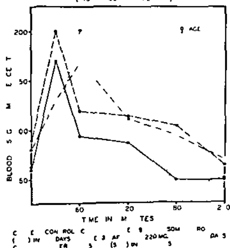


Fig 10 R V Glucose and Insulin Tolerance Tests Control and after STH Lot R491132



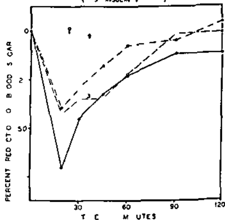


Fig 11 R V Glucose and Insulin Tolerance Tests Control and during STH Lots R491276 R527026B R527093 and R527093C

control value of 197 per mm³ There was a 12 per cent elevation of the basal metabolic rate above the control level

During the period over which these 4 lots of STH were given the subject developed an impaired glucose tolerance and increased insulin resistance (Fig 11)

The effects of various dose levels of STH were explored during the last year of study (Fig 12) Two lots of hormone were given at daily dosages

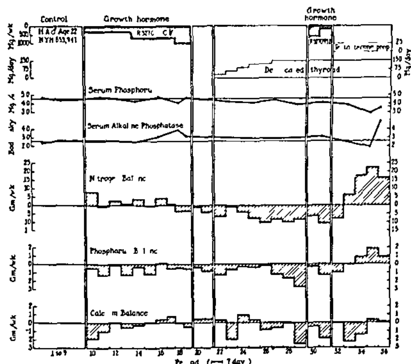


FIG 13 H A (m) 22 yrs 4 mos On constant daily intake of 1 802 calories 80 4 g protein (12 9 g nitrogen) 1 747 g phosphorus and 1 500 g calcium During the 9 week control period the subject was in positive balance with respect to nitrogen of 6 16 phosphorus of 2 611 and calcium of 1 967 g per week

After a 9 week control period he received for 10 weeks Lot R527093C of STH which had previously induced anabolic effects in subject R V The dosage schedule was successively 50 100 and 200 mg per day (Fig 13) An equivocal intermittent anabolic effect on nitrogen metabolism resulted with no enhancement of phosphorus and calcium retention There was no post treatment rebound The serum inorganic phosphorus was not elevated and the alkaline phosphatase showed a maximal rise of 1 2 B U During the last 2 periods the eosinophil count was 1 227 per mm³ Because of thyroid insufficiency he was then placed on ascending doses of desiccated thyroid to a maintenance dose of 150 mg Following a 10 week period of thyroid therapy he was begun on STH R527093B a lot which R V had received for 12 weeks without any hypersensitivity reactions After 1 500 mg had been given over a period of one week he had a sudden acute hypersensitivity reaction immediately following an injection This consisted of hypotension cyanosis urticaria lacrimation dyspnea abdominal cramps diarrhea tachycardia chills and fever Rapid relief was obtained by an intravenous antihistaminic Ten days later he was skin tested with a new lot

basis of our vaginal smear assays in the human In addition she received anhydro hydroxyprogesterone 100 mg daily during the fifth period to induce shedding of the endometrium which occurred two days after stopping medication The bleeding was of 5 days duration The amounts of estrogen given produced 90–100% cornification of the vaginal smear The concurrent metabolic changes were an unexpected enhancement of nitrogen and phosphorus storage and a very minimal increase in calcium retention There was a progressive fall in serum inorganic phosphorus to a low of 2 8 mg compared to the average control of 4 4 mg per cent There were no changes in the levels of urinary excretion of 17 ketosteroids or 11 oxysteroids

During the five months since her discharge she has been receiving 5 mg of Premarin® for 21 days of each month She has had withdrawal bleeding for 4–5 days commencing 7 days after therapy is discontinued In this interval her height has increased 0 7 cm

The third subject H A (m) N Y H 653941 was 22 years 4 months old at the time the study was begun in 1953 His growth and development were normal until the age of 10 between the 10th and 14th year there was a marked decrease in the linear growth rate At 15 treatment with Anteron® caused some growth of pubic hair At 17 a craniopharyngioma was discovered by 19 papilledema had developed and was treated with the removal of fluid from the cyst by needling and by X ray therapy He was asymptomatic for 18 months prior to admission except for lack of perspiration He had had occasional erections no emissions no facial or axillary hair His parents and 4 siblings measured 165–180 cm in height

Physical Examination on Admission Weight 41 60 kg height 149 6 cm, sitting height 75 4 cm arm span 149 8 cm The bodily contour was symmetrical There were a few pubic hairs and no facial or axillary hair Slight temporal pallor of the optic discs was present The penis was small and prepuberal both testes were palpable and the prostate was small Neurological examination was within normal limits Blood pressure was 92/60

Laboratory Data on Admission *Visual Fields* bilateral left homonymous defect *X ray of Skull* suprasellar calcification with no enlargement of the sella *Bone age* 14 years (Todd s standards) *Thyroid Status* B M R minus 23% serum cholesterol 364 mg % serum protein bound iodine 4 6 gamma % I^{131} neck uptake 42 8% and excretion 55 5% in 48 hours *Adrenal Status* serum electrolytes within normal limits eosinophils 121 per mm³ with an 82% fall following a 4 hour intramuscular ACTH test normal response to 6 day salt withdrawal test urinary 17 ketosteroids 4 20 mg and 11 oxysteroids 1 80 mg per 24 hours mild insulin hypersensitivity (see below) *Gonadal function* urinary gonadotropins positive at 6 5 m u negative at 13 m u *Carbohydrate* glucose tolerance F B S 67 30 min 112, 60 min 100 120 min 78 180 min 101 240 min 98 mg % insulin tolerance F B S 62 20 min 22 30 min 18 45 min 45 60 min 59 90 min 62 120 min 65 mg %

tative measurement of the relative amounts of protein present in the precipitate was obtained (Fig 14) The striking increase in the amount of protein precipitated from the patient H A is taken as evidence of the presence of a precipitin to STH in his serum There is excellent agreement between the STH blanks and the protein precipitate in the normal control and in R V who had tolerated many lots of STH with only minor reactions

The patient then received daily injections of testosterone propionate in the amount of 25 mg intramuscularly for 5 weeks There resulted a marked increase in the retention of nitrogen and phosphorus with no added retention of calcium The serum inorganic phosphorus fell 1 6 mg % and alkaline phosphataste rose 2 4 B U maximally

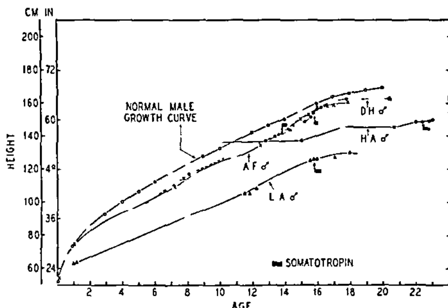


FIG 15 Growth curves of the male subjects A F D H L A and H.A

During STH therapy the subject s weight fell 1 52 kg his height increased 0 7 cm The bone age remained unchanged (Fig 15) In the 5 months following his discharge during which time he was maintained on desiccated thyroid and methyl testosterone (50 mg daily) his height had increased by 1 0 cm and weight by 6 7 kg

The other three subjects studied A F D H and L A were all males Their endocrine status is summarized in Table 1 They were all prepuberal at the time they were studied and all had some axillary and pubic hair Thyroid function was normal in all three There were no derangements in carbohydrate metabolism in A F and D H whereas L A was insulin sensitive Their growth curves are given in Figure 15 Of all the subjects in this series A F and D H showed the least difference 8 8 cm and 5 3 cm

of STH D674010, 50 gamma per ml intracutaneously produced a flare and 500 gamma per ml a flare wheal and pseudopods The conjunctival reaction was negative to 500 gamma per ml The patient was then skin tested with the lot to which he had such a profound hypersensitivity response as was subject R.V and four normal controls H A again showed a flare at 50 gamma and a flare wheal and pseudopods at 500 gamma per ml none of the others had a positive reaction All conjunctival tests again were negative

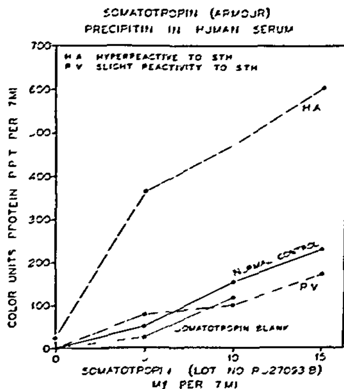


Fig 14

The sera of this patient of R.V and of one control were tested for antibodies to STH using Lot R527093B as the antigen The STH was dissolved in normal saline and the clear supernatant employed for precipitin tests The exact amount of STH in the antigen solution was not known A series of tubes were set up which contained 1 ml of serum and increasing amounts of STH and brought to a constant volume of 7 ml with normal saline After mixing, the tubes were incubated at 37 C for one hour and then kept at 4 C A precipitate formed within 24 hours and after standing for one week was spun down in a refrigerated centrifuge and washed 3 times with saline The tyrosine tryptophane content of the precipitates was then determined by the method of Heidelberger and MacPherson[20] By this means a quanti

tative measurement of the relative amounts of protein present in the precipitate was obtained (Fig 14) The striking increase in the amount of protein precipitated from the patient H A is taken as evidence of the presence of a precipitin to STH in his serum There is excellent agreement between the STH blanks and the protein precipitate in the normal control and in R V who had tolerated many lots of STH with only minor reactions

The patient then received daily injections of testosterone propionate in the amount of 25 mg *intramuscularly for 5 weeks There resulted a marked* increase in the retention of nitrogen and phosphorus with no added retention of calcium The serum inorganic phosphorus fell 1 6 mg % and alkaline phosphataste rose 2 4 B U maximally

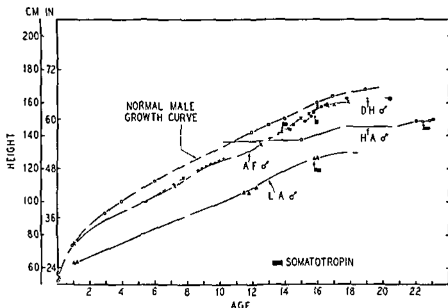


FIG 15 Growth curves of the male subjects A F D H L A and H A

During STH therapy the subject s weight fell 1 52 kg his height increased 0 7 cm The bone age remained unchanged (Fig 15) In the 5 months following his discharge during which time he was maintained on desiccated thyroid and methyl testosterone (50 mg daily) his height had increased by 1 0 cm and weight by 6 7 kg

The other three subjects studied A F, D H and L A were all males Their endocrine status is summarized in Table 1 They were all prepuberal at the time they were studied and all had some axillary and pubic hair Thyroid function was normal in all three There were no derangements in carbohydrate metabolism in A F and D H whereas L A was insulin sensitive Their growth curves are given in Figure 15 Of all the subjects in this series A F and D H showed the least difference 8 8 cm and 5 3 cm

respectively between actual height and the mean for their ages in D H there was an associated mild osteochondrodystrophy On the other hand the height of L A who had two dwarfed cousins was 23 cm below the mean for his age All three differed from the other subjects in that their bone ages were essentially normal for their chronological age according to Todd s standards

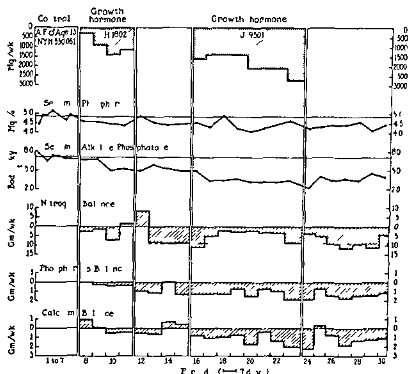


FIG 16 A F (m) 13 yrs 7 mos On constant daily intake of 2 227 calories 84 6 g protein (13 5 g nitrogen) 1 522 g phosphorus and 1 503 g calcium During the 7 week control period the subject was in positive balance with re spect to nitrogen of 12 53 phosphorus of 2 121 and calcium of 2 989 g per week The interrupted line represents the new control baseline for calculating the effects of STH on the nitrogen balance for periods 13 through 30 (see text)

Subject A F was studied for 8 months during which he received two lots of Armour STH (Fig 16) The first lot given H1802 was selected because it had induced anabolic effects in subject L F He received 3 675 mg over a period of 28 days with an average daily dose of 131 mg and a maximum of 200 mg The nitrogen balance became slightly negative with a positive rebound for the week after STH was stopped and then fell to well below the original baseline where it persisted for the 3 weeks prior to the adminis tration of the second lot of STH J9501 This lot was given over a period of 62 days in a total amount of 14 800 mg with an average daily dose of 239 mg and a maximal of 300 mg The assessment of the effects of this lot

of STH is dependent on the control baseline selected for comparison Several features of the metabolic data would seem to justify the choice of the new baseline for nitrogen metabolism established by this subject for the 3 weeks (periods 13–15) prior to the administration of the new lot of hormones rather than the original control values (periods 1–7) Most relevant is the fact that the nitrogen balance returned to the new baseline after the STH administration was discontinued instead of reverting to the original control value Furthermore both the phosphorus and calcium balances also fell to and persisted at lower levels during these latter portions of the study Assuming this new baseline Lot J9501 induced an anabolic effect on nitrogen metabolism during 46 of the 62 days of its administration The total extra storage of nitrogen amounted to 34 72 g with an average of 3 92 g per week for the entire period of treatment and a maximum of 6 37 g during the third and sixth week of STH administration There were no consistent parallel alterations in calcium and phosphorus balances except for the decline of both to lower levels Attention should be called to the lower levels of serum inorganic phosphorus and alkaline phosphatase which prevailed after the initiation of STH administration No consistent or significant alterations were noted in the other numerous indices studied While receiving both lots of STH this subject developed urticaria without eosinophilia which was readily controlled with antihistaminics This subject was followed until the age of 18 years He underwent a normal somatic and sexual development and a steady increase in height to 160 5 cm still 8 5 cm below the mean for his age His bone age and skeletal development were normal for his years

Subject D H was under study for a period of 6 months during which he received Armour STH Lot J9501 This preparation proved anabolic in subject A F who received a total of 14 300 mg for 8 weeks with a daily average and maximal dose of 255 and 400 mg respectively No significant alterations resulted in any of the metabolic indices However during the fourth week after STH had been discontinued there occurred a spontaneous increase in nitrogen storage which persisted for the last 3 weeks of the study and averaged 22 2 g per week There was a concomitant rise in urinary 17 ketosteroids from the previous average level of 3 31 mg to 8 60 mg per 24 hours This was regarded as a reflection of the onset of the puberal spurt in sexual development The only side reaction noted to STH was the rise in the eosinophil count from the control level of 314 to 1132 mm^3 during therapy When last seen at the age of 20 years 6 months he had reached a height of 163 cm still 9 7 cm below the mean for his age His somatic and sexual development were that of a normal adult male and his epiphyses were closed

The last subject L A received two lots of Armour STH Lot J17710R was given over a period of 96 days for a total of 15 125 mg with an average and maximal daily dose respectively of 158 and 300 mg For the

second trial Lot J21609R previously anabolic in subject R.V. was given for 14 days for a total of 2,285 mg with an average daily dose of 202 and a maximum of 300 mg. During both therapeutic periods no significant changes were noted in any of the metabolic indices. At 18¾ years of age, his height was 130 cm 40.7 cm below the mean for his age. His weight was 37.4 kg. His bone age was normal. His left testis was small, the right atrophic. Gynecomastia was present. He reported few erections and emissions. There was only a moderate growth of axillary and pubic hair. He shaved twice weekly.

Discussion

From the data obtained in this study it would seem permissible to draw at least three conclusions with regard to the action of somatotropin in human subjects. The first and most important is that crystalline somatotropin as prepared by the Armour Laboratories by the method of Wilhelmi, Fishman and Russell is capable of bringing about many of the major metabolic effects characteristic of the action of this hormone in animals (Table 2). These include the enhancement of the storage of nitrogen, phosphorus and calcium requisite for the growth of the skeleton and soft tissues as well as impairments in carbohydrate metabolism manifested by the development of a diabetic type of glucose tolerance curve, increased insulin resistance and glycosuria. The second conclusion is that these metabolic effects are not uniformly produced by STH in all subjects and the third that there is no present explanation for these inconsistencies.

Table 1

ENDOCRINE STATUS OF SUBJECTS RECEIVING SOMATOTROPIN

Subject Sex	Height / Mean	Age / Bone Age	Thyroid Function	Gonadal Function	Carbohydrate	Electrolytes	Miscellaneous
L F (F)	136.6 / 158.0	14 / 10	N	Pubertal	Insulin sensitive	N	
R V (F)	124.8 / 161.0	18 / 11	↓	Absent	Insulin sensitive	N	Platybasia
A F (M)	141.2 / 150.0	13 / 12	N	Pubertal	N	N	
D H (M)	152.7 / 158.0	15 / 14	N	Pubertal	N	N	Osteochondrodystrophy mild
L A (M)	125.0 / 158.0	15 / 16	N	Pubertal	Insulin sensitive	N	2 dwarf cousins
H A (M)	149.4 / 175.0	22 / 13	↓	Absent	Insulin sensitive	N	Craniopharyngioma

Table 2

COMPARISON OF ACTION OF SOMATOTROPIN IN ANIMALS AND MAN

Index	Animals	Man
Nitrogen	Increased retention	Increased retention
Calcium	Increased uptake and turnover in bone	Increased retention
Phosphorus	Increased uptake and turnover in bone	Increased retention
Serum Inorg P	Elevated	No consistent change
Serum Alk P-ase	Elevated	No consistent change
Glycosuria	Present reversible or permanent	Present reversible
Glucose Tolerance	Impaired diabetic type reversible or permanent	Impaired diabetic type reversible
Insulin Resistance	Increased	Increased reversible

In the 2 subjects L F and R V in whom STH produced unequivocal anabolic effects the magnitude of the storage of nitrogen calcium and phosphorus is impressive In Table 3 the data are summarized for subject L F and for periods 7 through 20 for subject R V (Fig 9) With L F who received a total of 9 38 g of STH in 11 weeks the anabolic effect persisted for 18 weeks in all with a total retention of 149 5 g of nitrogen 17 2 g of calcium and 13 7 g of phosphorus The extra nitrogen storage is equivalent to 4 6 kg. of protoplasm During the same interval there was a proportional increase in urinary creatinine suggestive of the incorporation of the bulk of the stored nitrogen in the muscle mass In the subject R V

Table 3

MAGNITUDE OF SOMATOTROPIN EFFECT

ON NITROGEN CALCIUM & PHOSPHORUS METABOLISM

Patient	Total STH	Duration of Treatment	Duration of Action	Total extra retention of N_2	Total extra retention of Ca	Total extra retention of P
	gm	weeks	weeks	gm	gm	gm
L F	9 38	11	18	149 5	17 2	13 7
R V	3 45	3	14	78 8	11 4	17 0

3 45 g of STH given over 3 weeks induced anabolic effects which persisted for 14 weeks in all with a total extra retention of 78 8 g of nitrogen (equivalent to 2 4 kg of protoplasm) of 11 4 g of calcium and 17 0 g of phosphorus There is no apparent explanation for the persistence of the anabolic effects for such long periods as 7 and 11 weeks after the hormone was discontinued It may represent the carryover of metabolic processes set in motion during the period of STH administration or the continued action of STH precipitated at the injection sites and only slowly released from these depots into the circulation The latter possibility is suggested by the solubility characteristics of the hormone The Armour preparations used in this study are injected at pH 9 3 at which they are completely soluble Precipitation may well occur at the physiological pH of the injection site prior to slow re solution in the circulating fluids

Of particular interest also was the production in both these subjects of carbohydrate changes of anti insulin nature The development of impaired glucose tolerance increased insulin resistance and in subject L F of frank glycosuria is analogous to the changes in carbohydrate metabolism observed in animals with this hormone The altered carbohydrate metabolism in both subjects reverted to the pretreatment status when treatment was discontinued The diabetogenic action of crystalline STH prepared by either the method of Li or of Wilhelmi et al has been demonstrated in the cat and the dog De Bodo and his associates [31] working with hypophysectomized dogs observed the development of insulin resistance and impaired glucose tolerance even in the absence of the adrenal cortex Although these findings are considered by most observers to be due to STH per se rather than to some hormonal contaminant Raben and Westermeyer[3] on the basis of their work with a STH prepared by their procedure believe that the carbohydrate effects can be divorced from the growth promoting properties Our data do not contribute to the resolution of these conflicting views the failure in subject R V of these carbohydrate changes to occur in every instance in which STH induced storage of nitrogen must be regarded as inconclusive with respect to the anti insulin action being an inherent property of STH

In spite of these clear cut demonstrations of the anabolic properties of STH in 2 of the 6 subjects and the probability of a similar but less marked effect in 2 other subjects it is apparent from our data and those of other investigators that STH preparations with equal growth promoting properties in animals are not equally or consistently effective in achieving nitrogen phosphorus or calcium storage or alterations in carbohydrate metabolism in man Some of these discrepancies may be methodological e g too brief treatment or inadequate dosage In our studies anabolic effects occasionally did not occur until after the first or second week of treatment and the most striking effects were obtained with an average dosage of from 112 to 218 mg daily yet equally high doses and prolonged therapy were ineffective in other subjects Side effects particularly pyrogenic reactions which have

been described by other workers, cannot be held responsible for the lack of effect in our unresponsive subjects since they were rarely encountered The development of eosinophilia was unrelated to the responsiveness to the hormone The possibility of anti hormone formation cannot be excluded but is regarded as doubtful One subject H A (Figs 13 and 14) who exhibited an equivocal nitrogen storage during his first course of STH developed precipitins in his serum when given STH for the second time along with a profound hypersensitivity reaction to the hormone Yet R V who had received 11 lots of STH over a 4 year period had no more precipitins in her serum than did a normal untreated control

These discrepancies could also be due to variable contamination of crystalline STH with other anterior pituitary factors with activities which might mask or counteract those of STH The assay data on the Armour STH preparations used in the present study are given in Table 4 and are based on assays carried out in the Armour Laboratories These preparations were virtually free of ACTH which would make it unlikely that the rise in

Table 4

ASSAY DATA ON SOMATOTROPINS (ARMOUR) USED IN PRESENT STUDY

Lot No	TSH USP u t/mg	Met bol Effe ts Po t	N gat
G411	0 08	L F (f)	
H1802 } H1902 }	0 06	L F (f)	A F (m)
J9501	0 08	A F (m)±	D H (m)
J17710R	0 05		R V (f) L A (m)
J21609R	0 04	R V (F)	L A (m)
K43806R	0 04	R V (F)	
K90407R	0 06	R V (f)	
R491132	0 04	R V (f)	
R491276	0 01	R V (f)	
R5270268	<0 06	R V (f)	
R527093	0 02	R V (f)	
R527093C	0 02	R V (f) H A (m)_	
R5270938	0 02	R V (f)_	H A (m)
5271208	0 02	R V (f)	

All Amour Somatot op ? pa t o to ned (tho 1 0 u t I p te or p t to y o t ty pe 50 mg and no d t tabl ACTH or gonadot op

17 ketosteroids and 11 oxysteroids in L F, towards the end of her course of treatment with STH, could be attributed to contamination with this hormone All lots of STH contained variable amounts of thyrotropin ranging from 0 01 to 0 08 USP unit per mg however it was not possible to correlate the differences in the anabolic activity of different lots of STH with the extent of thyrotropin activity As regards the presence of posterior pituitary activity this did not lead to any significant alterations in water balance in any of our subjects

There remains for consideration the possibility that responsiveness to the hormone might be conditioned by the metabolic status of the subject At the time of the study 3 of the males (A F D H and L A) had no discernible endocrine abnormalities associated with their short stature L A failed to respond to a lot of STH which was anabolic in the subject R V and A F to a preparation which was very effective in subject L F although A F did have what we regard as an anabolic response to another lot of hormone which in turn was without effect in subject D H Hence there was only one positive response in these 3 essentially normal boys Nevertheless, a striking anabolic effect was obtained in the young girl L F who was essentially normal except for short stature and a delayed bone age In connection with the endocrine status conditioning the response in man it should be pointed out for future reference that R V when in the hypothyroid state failed to respond to a STH preparation which produced anabolic effects when the thyroid insufficiency was corrected An opportunity in the case of the male, H A , to further test this possible relation of thyroid function to STH action was precluded by his development of hypersensitivity to the hormone when it was given after correction of his thyroid insufficiency We are forced to conclude that the available data are inadequate to reveal any clear relationship between the metabolic and endocrine status of the subject and responsiveness to STH

The data concerning the changes in height and weight after STH are included for the sole purpose of registering the information together with the opinion that they do not permit any conclusions as to the relation between the changes and the STH administered

Summary and Conclusions

Somatotropin (STH) prepared by the Armour Laboratories according to the method of Wilhelmi Fishman and Russell was administered to 6 human subjects 4 males and 2 females of abnormally short stature Marked enhancement of nitrogen calcium and phosphorus storage was observed during the administration of various lots of STH to the 2 females as well as anti insulin effects evident in the development of impaired glucose tolerance increased insulin resistance and in one of the girls frank glycosuria These latter effects reverted to the pretreatment status after STH was discontinued A modest increase in nitrogen storage was obtained in

one of the male subjects an equivocal response in a second The other 2 males were unresponsive to the hormone Hypersensitivity to STH developed in one of the males in association with increased precipitins to STH in the serum

These findings in man are confirmatory of similar actions of STH previously reported to occur in the dog and cat However these effects are produced with less constancy in the human subject a phenomenon for which there is no present explanation

References

1 Evans H M and J A Long *Anat Rec* **21** 62 (1921)
2 Li C H Evans H M and M E Simpson *J Biol Chem* **159** 353 (1945)
3 Wilhelmi A E Fishman J B and J A Russell *J Biol Chem* **176** 735 (1948)
4 Bennett L L Weinberger H Escamilla R Margen S Li C H and H M Evans *J Clin Endocrinol* **10** 492 (1950)
5 Lewis R A Klein R and L Wilkins *J Clin Invest* **29** 460 (1950)
6 Carballeira A Elrick H Mackenzie K R and J S L Browne *Proc Soc Exp Biol Med* **81** 15 (1952)
7 Raben M S Westermeyer V W and A Leaf *J Clin Invest* **31** 655 (1952)
8 Conn J W Fajans S S Louis L H and H S Seltzer *J Lab Clin Med* **40** 788 (1952)
9 Escamilla R F and L Bennett *J Clin Endocrinol* **11** 221 (1951)
10 Crispell K R and W Parson *J Clin Endocrinol* **12** 881 (1952)
11 Kinsell L W Balch H E and G D Michaels *Proc Soc Exp Biol Med* **83** 683 (1953)
12 Kinsell L W Margen S Partridge J W Michaels G D Balch H E and J P Jahn *J Clin Endocrinol* **14** 110 (1954)
13 Crispell K R Parson W and G Hollifield *J Clin Invest* **33** 924 (1954)
14 Gephart F C and E F Du Bois *Arch Int Med* **15** 829 (1915)
15 Du Bois E F *Methods and Problems of Medical Education* 1928
16 Deitrick J E Whedon G D and E Shorr *Am J Med* **4** 3 (1948)
17 Sherman H C *Chemistry of Food and Nutrition* 6th ed New York The Macmillan Company 1941
18 Bills C E McDonald F G Niedermeier W and M C Schwartz *J Am Dietet Assoc* **25** 304 (1949)
19 Daughaday W H Jaffe H and R H Williams *J Clin Endocrinol* **8** 166 (1948)
20 Gorbman A *Endocrinology* **37** 177 (1945)
21 Smith P H Albright F and E Dodge *J Lab Clin Med* **28** 1761 (1943)
22 Gutman A B Personal communication
23 Schales O and S S Schales *J Biol Chem* **140** 879 (1941)
24 Van Slyke D D and G E Cullen *J Biol Chem* **30** 289 (1917)
25 Hawk P B Oser B L and W H Summerson *Practical Physiological Chemistry* 12th ed New York The Blakiston Co 1947 531
26 Barker S B Humphrey M J and M H Soley *J Clin Invest* **30** 55 (1951)

27 Nelson N *J Biol Chem* **153** 375 (1944)
28 Thorn G W Forsham P H Prunty F T G and A G Hills *J A M A* **137** 1005 (1948)
29 Shorr E *J Mt Sinai Hosp* **12** 667 (1945)
30 Heidelberger M and C F C MacPherson *Science* **97** 405 (1943)
31 DeBodo R C and M W Sinkoff *Ann N Y Acad Sci* **57** 23 (1953)
32 Raben M S and V W Westermeyer *Proc Soc Exp Biol Med* **80** 83 (1952)

DISCUSSION

Influence of Growth Hormone on the Mammary Gland and on Human Metabolism

Designated Discussion

FRANCIS MOORE (Harvard Medical School) In comparison with the experiments of Dr Kinsell and Dr Shorr ours are very few and very recent Our interest in growth hormone as it relates to the studies of surgical convalescence arises from 4 rather distinct considerations Firstly the spontaneous anabolic phase of surgical convalescence, when nitrogen is being added to the body at a steady high rate at a fairly low calorie to nitrogen ratio and at a low potassium to nitrogen ratio bears certain resemblances to the normal growth of children It is a very interesting and extremely important phase of surgical convalescence remembered by any former patient as the period when he felt well again and regained his strength and vigor We have sought therefore for growth promoting substances in the serum of such patients, using the bioassay techniques discussed during the first day of this meeting Our experience is very small to date but in one patient recovering from burns we have identified a positive result

Secondly a growth hormone or a growth promoting substance would be most useful if it could produce anabolism in the face of the low caloric intakes characteristic of the surgical convalescent who is unable to eat normally for one reason or another We have studied this aspect as you will see in a moment without finding at least the expected results

Thirdly, and taking a leaf from Dr Selye s experiment which he described at this meeting growth hormone appears to antagonize certain peripheral actions of ACTH and the adrenal steroids It would be interesting, therefore if growth hormone or a substance of this type might modify the early post traumatic metabolism which is so suggestive of ACTH and steroidal effects Would such a substance dampen or modify the so-called stress response in man under the rare circumstances when the latter may be deleterious to the individual?

And fourthly growth hormone or the substances which we have been discussing at this meeting produce connective tissue changes in the periphery which resemble the fibrosis of wound healing and for that reason also they are of interest to surgery

In the accompanying figures I will present data from one or two of the metabolic studies In Figure 1 is shown a typical metabolic study carried out on a patient convalescing from an abdominal operation He was several days post operative when the study was begun but was still in negative nitrogen balance The metabolic balances are plotted such that the intake is charted up from the zero line and the output downward from the top of the intake line so that a negative balance is below the zero line and a positive balance is above The data in Figure 1 is perfectly characteristic of a post operative metabolic response We would expect the patient at about the 5th or 7th day to reach positive nitrogen balance associated with the in creased caloric intake However in this individual we held his caloric intake purposely at a low level to see if growth hormone would produce by any chance an anabolic effect under the suboptimal caloric conditions He was given the material intravenously 200 mg daily for 4 days There was no effect on nitrogen balance and possibly a slight effect on potassium balance There appears to have been a tendency to retain sodium which effect might be related to some interesting actions of growth hormone which I under stand will be discussed by Dr Beck However there was no change in weight Blood eosinophil levels did not change significantly and urinary 17 hydroxycorticoids measured by the method of Reddy Jenkins and Thorn likewise did not change during the two periods when the hormone was given This observation is in contrast to the findings in one of the other patients I will mention The growth hormone was then stopped and the patient was permitted to enter spontaneous anabolism with an increased caloric intake At this time the hormone was given again but it is important to realize that we had a much different metabolic situation and that surgical convalescence in itself is a very dynamic process which changes each day The hormone again had no very clear-cut effect on any metabolic depart ment except to reverse the previous slight tendency to retain sodium

In another case (Fig 2) we studied a volunteer patient as a normal control over a period of about one month He was given injections of a Raben Westermeyer growth hormone preparation kindly supplied to us by Dr Astwood s group The drop in the eosinophil level was probably sig nificant although surely this index is most fickle The changes in urinary 17 ketosteroids were not considered significant but there was a very striking increase in the urinary 17 hydroxycorticoids Likewise determinations of serum 17 hydroxycorticoids by the method of Nelson and Samuels revealed a very significant increase Our interpretation would have been that this preparation was contaminated with ACTH inasmuch as the patient was not stressed i e he did not have a febrile or other reaction to the material We thought this type of response might be peculiar to that sample of hormone supplied to us by Dr Raben We have since worked with growth hormone material from the Armour Laboratories which study has all been done in the past year

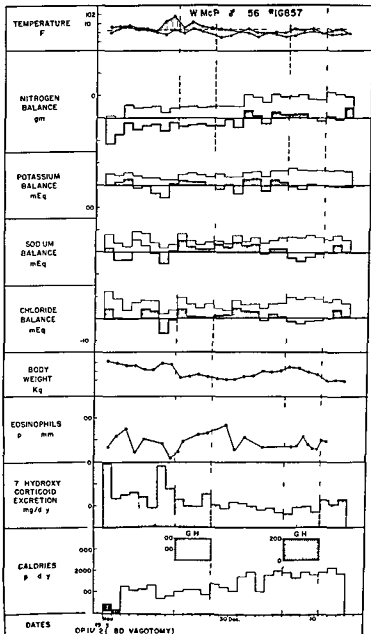


FIG 1 Metabolic Chart Analyses of nitrogen potassium sodium and chloride are charted as described in the text together with body weight eosinophil count 17 hydroxycorticoid excretion and caloric intake The administration of growth hormone at two different phases in normal convalescence appears to have produced little measurable effect on any index studied

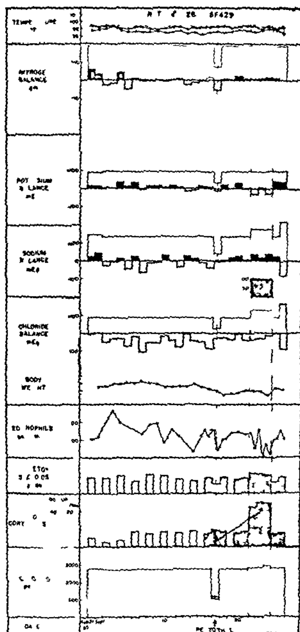


FIG 2 Metabolic Balance Study in a Normal Volunteer Charted as in Figure 1 The administration of Raben Westermeyer Growth Hormone 100 mg per day for three days produced a definite fall in eosinophils and rise in urinary excretion of 17 hydroxycorticoids The solid line in the 17 hydroxycorticoid chart is the blood concentration of the free steroid There was a definite increase of both blood and urine hydroxycorticoid during G H treatment

A few days prior to the growth hormone administration a pentothal anesthesia was given as part of a separate study It did not appear to influence subsequent metabolic events

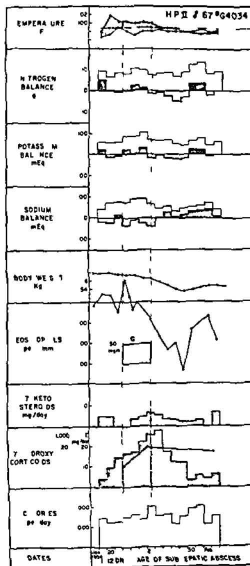


FIG 3 Metabolic Balance Chart of Patient Receiving Growth Hormone in Convalescence as in Figure 1 Here there was a definite increase in urinary excretion of 17 hydroxycorticoids but no other significant changes were noted save for a slight tendency to gain potassium and lose sodium

Metabolic data from another patient is shown in Figure 3 This study was made in the postoperative period and on the 9th day he was started on 300 mg of growth hormone intravenously daily for 5 days We started on 300 mg of growth hormone intravenously daily for 5 days We observed no change in his 17 ketosteroid excretion and nothing happened metabolically which we are able to call significant There was a very definite increase however in urinary 17 hydroxycorticoids and a slight increase in the serum 17 hydroxycorticoids The method employed is very sensitive in

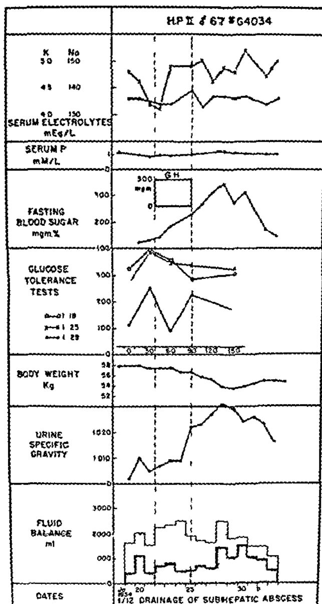


FIG 4 Same Patient as in Figure 3 This demonstrates the rise in fasting blood sugar during and after Growth Hormone and the change in glucose tolerance The glucose tolerance test marked with the solid black dots was done before the growth hormone was given and the tests marked in open circles and with the X were done after the growth hormone was given

our experience, and it reflects adrenal changes in response to very minimal amounts of trauma or ACTH The steroid increases suggest that a significant amount of ACTH or ACTH like material accompanied the growth hor mone The patient mildly diabetic, was the only subject in whom we have observed growth hormone induced alterations in glucose metabolism His fasting blood sugar taken each morning rose consistently during the period of hormone administration as seen in Figure 4 Intravenous glu-ose tolerance tests performed before during and immediately after the course of hormone administration gave evidence of a progressive further decrease in his glucose tolerance This patient was the only one in whom we have seen evidence of diabetogenic effects There were no changes in serum phosphorus values

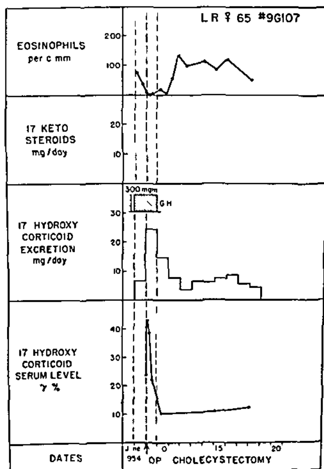


FIG 5 Endocrine chart demonstrating the early effect of growth hormone on the stress response to cholecystectomy There was no effect on the eosinophil count the urinary excretion of 17 hydroxycorticoids or the serum level of the free steroids The response in all three measured indices was entirely normal for this type of surgery *There was no evidence of an inhibitory effect by growth hormone at this dose level and under these circumstances*

And lastly in Figure 5 are data from a typical experiment in our efforts to determine if growth hormone preparations will inhibit or dampen the metabolic manifestations of the so called alarm reaction The patient was in the hospital for a cholecystectomy and on the day before and on the day of surgery she received 300 mg of an Armour growth hormone preparation intravenously The values for the blood eosinophils reflected a normal stress response The 17 hydroxycorticoid excretion likewise indicated a normal response and the serum 17 hydroxycorticoid peak attained within a few hours of making the incision was also quite normal for this surgical stress

I would summarize our limited studies by saying that we have found an ACTH like action in some of the material used and that we have not discovered any particularly useful or significant effects of growth hormone action in surgical convalescence Ending on a philosophical note however it has seemed to me that a number of the speakers during these 3 days have been fortunate in their first few experiments and as experience grows we on the surgical side of things must continue to study the potentialities of this very interesting material even if our initial experiences are negative in regard to the expected effects

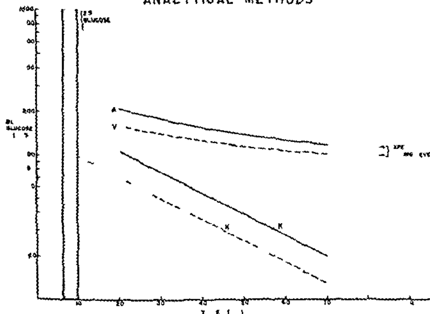


FIG 6 The upper curves show the logarithm of the actual capillary and venous blood sugar levels following the intravenous infusion of 25 g glucose The lower curves are plots of the difference between capillary and venous glucose levels and the experimental fasting levels derived from the upper curves

our experience, and it reflects adrenal changes in response to very minimal amounts of trauma or ACTH The steroid increases suggest that a significant amount of ACTH or ACTH like material accompanied the growth hormone The patient, mildly diabetic was the only subject in whom we have observed growth hormone induced alterations in glucose metabolism His fasting blood sugar taken each morning rose consistently during the period of hormone administration as seen in Figure 4 Intravenous glucose tolerance tests performed before during and immediately after the course of hormone administration gave evidence of a progressive further decrease in his glucose tolerance This patient was the only one in whom we have seen evidence of diabetogenic effects There were no changes in serum phosphorus values

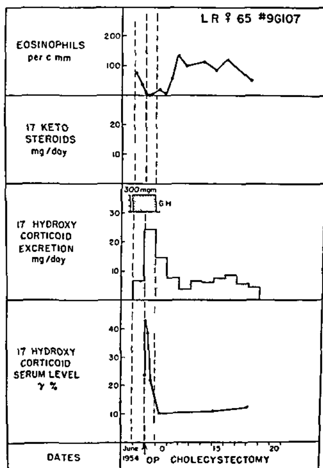


FIG 5 Endocrine chart demonstrating the early effect of growth hormone on the stress response to cholecystectomy There was no effect on the eosinophil count the urinary excretion of 17 hydroxycorticoids or the serum level of the free steroids The response in all three measured indices was entirely normal for this type of surgery There was no evidence of an inhibitory effect by growth hormone at this dose level and under these circumstances

HAROLD ELRICK (Veterans Administration Hospital and University of Colorado Medical School) I would like to present a summary of some of our investigations on the action of pituitary growth hormone in the adult human These findings are based on studies of 29 men using highly purified growth hormone which was kindly supplied by Dr Mitchell of Horner Ltd Canada and prepared from bovine or pork pituitary glands by the Wilhelmi or the Raben Westermeyer Astwood methods The complete papers (two) of which this is a summary will be published soon under the joint author ship of H Elrick T A Witten C J Hlad Jr G Clark T M Bow and A J Dukes Some of the initial experiments were done in collaboration with Drs A Carballeira K R Mackenzie and J S L Browne others were performed at Memorial Center New York City (unpublished data)

First I will briefly discuss our observations in regard to carbohydrate metabolism Early in our studies it was found that a single intravenous injection of growth hormone in doses ranging from 10 to 225 mg con

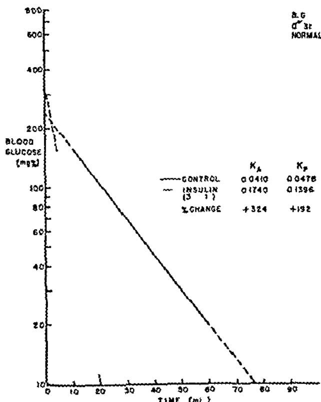


Fig 9 Note the marked increase in total and peripheral glucose assimilation when a small dose of insulin is added to the intravenous glucose infusion

ANALYTICAL METHODS

PERIPHERAL ASSIMILATION $\propto K_P \propto \frac{A-V}{A}$

TOTAL ASSIMILATION $\propto K_A$ = SLOPE

Fig 7 Indices used to measure glucose assimilation

THE EFFECT OF PGH UPON GLUCOSE ASSIMILATION

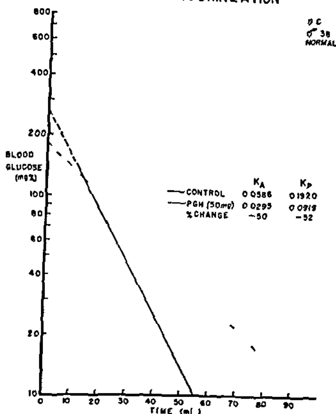


Fig 8 Note the marked decrease in total and peripheral assimilation of glucose following growth hormone infused over a period of 15 minutes 5 minutes before the glucose load

HAROLD ELRICK (Veterans Administration Hospital and University of Colorado Medical School) I would like to present a summary of some of our investigations on the action of pituitary growth hormone in the adult human These findings are based on studies of 29 men using highly purified growth hormone which was kindly supplied by Dr Mitchell of Horner Ltd Canada and prepared from bovine or pork pituitary glands by the Wilhelmi or the Raben Westermeyer Astwood methods The complete papers (two) of which this is a summary will be published soon under the joint authorship of H Elrick T A Witten C J Hlad Jr G Clark T M Bow and A J Dukes Some of the initial experiments were done in collaboration with Drs A Carballeira K R Mackenzie and J S L Browne others were performed at Memorial Center New York City (unpublished data)

First I will briefly discuss our observations in regard to carbohydrate metabolism Early in our studies it was found that a single intravenous injection of growth hormone in doses ranging from 10 to 225 mg con

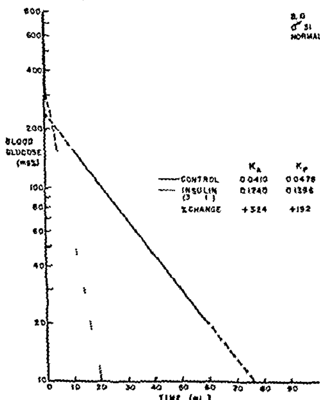


FIG 9 Note the marked increase in total and peripheral glucose assimilation when a small dose of insulin is added to the intravenous glucose infusion

sistently resulted in a hyperglycemia lasting 1½ to 3 hours with a maximum rise of 27 to 48 per cent above the fasting level The mechanism of this hyperglycemia was explored by means of a technique which combines a modified version of the intravenous glucose test of Amatuzio with simultaneous capillary venous glucose determinations (see Figs 6 and 7) The concentration of blood glucose after an intravenous glucose load (25 g infused in a period of 3 to 5 minutes) decreases as an exponential function of time and depends upon the rates of peripheral assimilation as well as upon the rates of hepatic assimilation and release of glucose Simultaneous capillary venous glucose differences can be used to derive an index of peripheral glucose assimilation as reported by Somogyi In this way, one has a method of assessing the effect of a given hormone or disease on the total (liver plus periphery) and peripheral assimilation of glucose

In the normal subject (Fig 8) a single intravenous injection of growth hormone results in a marked decrease in the rate of glucose disappearance

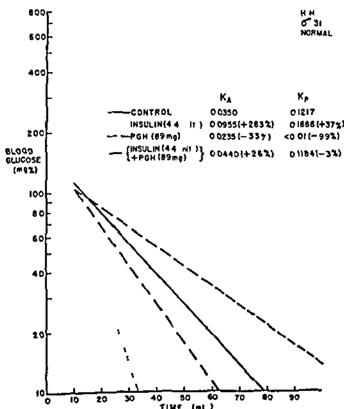


FIG 10 Note that the two hormones tend to cancel out the opposing action of the other on both total and peripheral assimilation

from the blood (total assimilation) as well as a decrease in its peripheral assimilation

As noted in Figure 9, the effect of glucagon free insulin (Lilly) is exactly opposite to that of growth hormone namely a marked increase in the rate of glucose disappearance from the blood and an increase in its peripheral assimilation

When both insulin and growth hormone are given during the same test period an effect is observed which is intermediate between either alone Such results as shown in Figure 10 are consistent with the concept that the two hormones oppose each other insofar as their action on glucose assimilation is concerned This phenomenon has been recently demon strated in the dog by both de Bodo and Bennett using two widely different techniques

The contribution of the liver to the action of growth hormone was

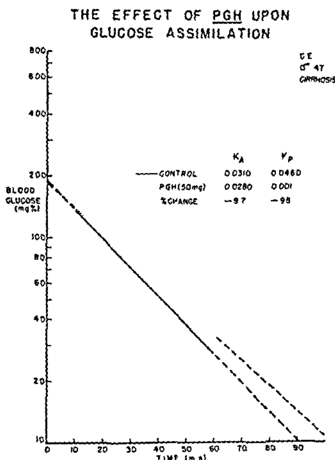


Fig 11 Note the absence of effect on total as imilation with the marked effect on peripheral glucose assimilation following growth hormone

sistently resulted in a hyperglycemia lasting 1½ to 3 hours with a maximum rise of 27 to 48 per cent above the fasting level The mechanism of this hyperglycemia was explored by means of a technique which combines a modified version of the intravenous glucose test of Amatuzio with simultaneous capillary venous glucose determinations (see Figs 6 and 7) The concentration of blood glucose after an intravenous glucose load (25 g infused in a period of 3 to 5 minutes) decreases as an exponential function of time and depends upon the rates of peripheral assimilation as well as upon the rates of hepatic assimilation and release of glucose Simultaneous capillary venous glucose differences can be used to derive an index of peripheral glucose assimilation, as reported by Somogyi In this way, one has a method of assessing the effect of a given hormone or disease on the total (liver plus periphery) and peripheral assimilation of glucose

In the normal subject (Fig 8) a single intravenous injection of growth hormone results in a marked decrease in the rate of glucose disappearance

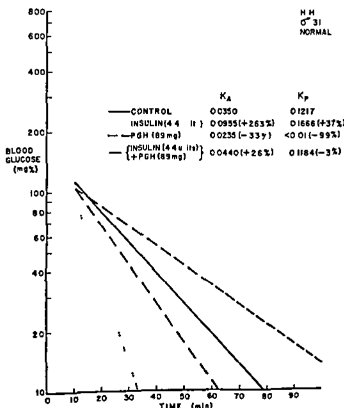


FIG 10 Note that the two hormones tend to cancel out the opposing action of the other on both total and peripheral assimilation

If this were true one would expect the effect of glucagon on glucose assimilation to be similar to that of growth hormone namely a depression of peripheral glucose assimilation From our data it is suggested that the effect of glucagon (Lilly) on the peripheral assimilation of glucose is directly opposed to that of growth hormone In Figure 13 it will be noted that glucagon causes a marked increase in peripheral glucose assimilation just as does insulin These observations are interpreted to suggest that not only are glucagon and insulin not antagonistic but that the two act in concert to maintain an adequate rate of peripheral glucose utilization The fact that these two agents have opposing actions on blood sugar and liver glycogen is entirely consistent with this concept

Briefly I will mention a few of our observations on the effects of growth hormone on amino acid metabolism During the early phase of our studies it was found that growth hormone speeds the removal of amino acids from the blood and decreases amino acid excretion following an intravenous

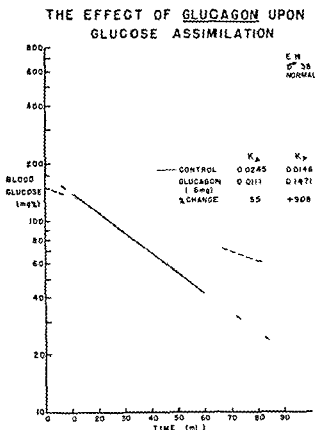


FIG 13 Note the marked increase in peripheral assimilation and the somewhat less marked decrease in total assimilation

investigated by studying patients with decompensated portal cirrhosis As depicted in Figure 11 the data reveal that the effect of growth hormone on peripheral glucose assimilation in the patient with severe liver disease is similar to that of the normal subject whereas the effect on total assimilation is significantly less We have found that the same holds true for the action of insulin

The role of the thyroid gland in the action of growth hormone on glucose assimilation was explored by studying a patient with severe myxedema (postoperative) From the results (Fig 12) we have concluded that the action of growth hormone in this patient was similar to that observed in the normal subject

Bornstein Reid and Young have presented some experimental evidence that growth hormone stimulates the release of glucagon (hyperglycemic factor) from the pancreas Since glucagon causes hyperglycemia and de creases liver glycogen it has been considered by some to be an insulin antagonist This suggests that the action of growth hormone on carbo hydrate metabolism might be mediated in part by the release of glucagon

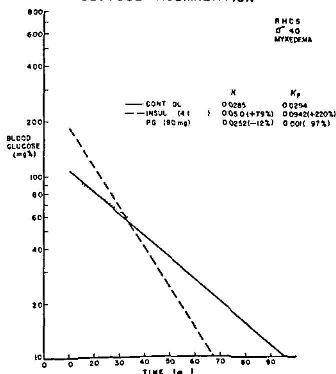


FIG 12 Note the marked decrease in peripheral glucose assimilation The decrease in total assimilation is much less marked The effect of insulin is similar to that seen in the normal subject

If this were true one would expect the effect of glucagon on glucose assimilation to be similar to that of growth hormone namely a depression of peripheral glucose assimilation From our data it is suggested that the effect of glucagon (Lilly) on the peripheral assimilation of glucose is directly opposed to that of growth hormone In Figure 13 it will be noted that glucagon causes a marked increase in peripheral glucose assimilation just as does insulin These observations are interpreted to suggest that not only are glucagon and insulin not antagonistic but that the two act in concert to maintain an adequate rate of peripheral glucose utilization The fact that these two agents have opposing actions on blood sugar and liver glycogen is entirely consistent with this concept

Briefly I will mention a few of our observations on the effects of growth hormone on amino acid metabolism During the early phase of our studies it was found that growth hormone speeds the removal of amino acids from the blood and decreases amino acid excretion following an intravenous

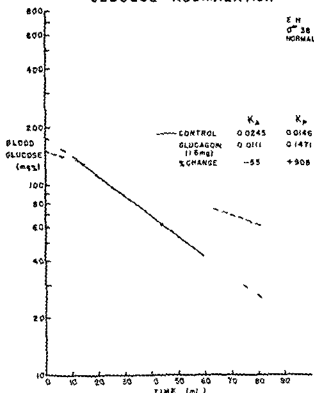


FIG 13 Note the marked increase in peripheral assimilation and the somewhat less marked decrease in total assimilation

FIG 14 Note the decrease in the maximal blood amino acid nitrogen level reached and the decreased excretion of amino acids following growth hormone as compared with the control experiment

infusion of synthetic amino acids This effect will be noted in Figure 14 In view of the fact that no increase in amino acid oxidation or increase in blood volume could be demonstrated it was felt that these results were consistent with the concept that amino acid retention had occurred as would be expected if protein synthesis were being stimulated Since the liver is a major site of protein synthesis one would expect that the accelerated clearance from the circulation and increased retention of amino acids observed in the normal subject following growth hormone injection would not be found in the patient with severe liver damage That such is the case can be seen in the data of Figure 15 and here it also will be noted that in the patient with severe myxedema the influence of growth hormone on amino acid assimilation and retention was similar to that observed in the normal subject

In summary I feel that our studies support the following concepts about the action of pituitary growth hormone in the adult human (Fig 16) (1)

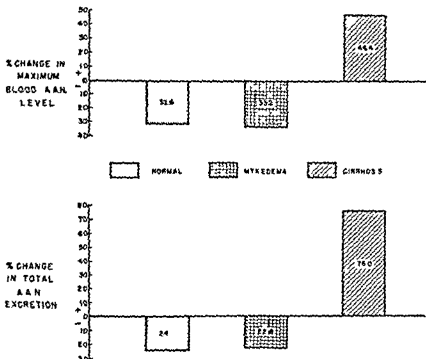


Fig 15 Note (a) the reversal of the effect on maximal blood amino acid nitrogen levels and urinary excretion in the patient with decompensated portal cirrhosis and (b) the normal response of the patient with myxedema as compared with data compiled from 5 normal subjects

THE ACTION OF PGH IN MAN

GLUCOSE ASSIMILATION

TOTAL

	NORMAL	MYXEDEMA	CIRRHOSIS
PGH	↓	↓	↓
INSULIN	↑	↑	—
GLUCAGON	↓		

PERIPHERAL

	NORMAL	MYXEDEMA	CIRRHOSIS
PGH	↓	↓	↓
INSULIN	↑	↑	↑
GLUCAGON	↑		↑

AMINO ACID ASSIMILATION AND RETENTION

	NORMAL	MYXEDEMA	CIRRHOSIS
PGH	↑	↑	↓

Fig 16 Diagrammatic summary of the action of growth hormone on glucose assimilation and amino acid metabolism and the comparison with the action of insulin and glucagon

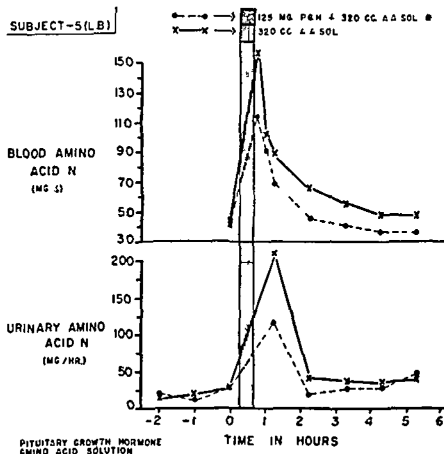


FIG 14 Note the decrease in the maximal blood amino acid nitrogen level reached and the decreased excretion of amino acids following growth hormone as compared with the control experiment

infusion of synthetic amino acids This effect will be noted in Figure 14 In view of the fact that no increase in amino acid oxidation or increase in blood volume could be demonstrated it was felt that these results were consistent with the concept that amino acid retention had occurred as would be expected if protein synthesis were being stimulated Since the liver is a major site of protein synthesis one would expect that the accelerated clearance from the circulation and increased retention of amino acids observed in the normal subject following growth hormone injection would not be found in the patient with severe liver damage That such is the case can be seen in the data of Figure 15 and here it also will be noted that in the patient with severe myxedema the influence of growth hormone on amino acid assimilation and retention was similar to that observed in the normal subject

In summary I feel that our studies support the following concepts about the action of pituitary growth hormone in the adult human (Fig 16) (1)

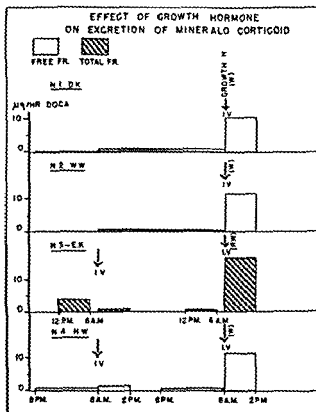


Fig 17

the sodium retaining factor Finally in the fourth patient the same effect held true These observations were made during some studies of the effect of growth hormone on the levels of plasma and urinary amino acid nitrogen It should be mentioned therefore that about 320 ml of an amino acid solution were infused both during the control and growth hormone periods

In a further study we changed our experimental procedure slightly We felt that the level of the 11 and 11 17 oxysteroids was highest in the morning period As many of you know these steroids may interfere with the bio assay of the sodium retaining factor For this reason we changed our experimental setup by dividing the urine collections into 3 periods of 8 hours each On the control day the patient received intravenously 200 ml of 5 per cent glucose in water and on the experimental day he received intravenously 100 mg of growth hormone dissolved in 200 ml of 5 per cent glucose and water As to the data of patient G A in Figure 18 you will note that there were no significant amounts of sodium retaining factor recovered during the control day while following the growth hormone

Growth hormone decreases total and peripheral glucose assimilation and is an antagonist to insulin in this respect Both of these hormones exhibit their action on peripheral assimilation but not on total assimilation in the presence of severe liver disease (2) The effect of growth hormone on glucose assimilation is not mediated through the release of glucagon since these two substances have opposing actions on the peripheral assimilation of glucose (3) The concept that growth hormone promotes protein synthesis is supported by our observations that it accelerates the assimilation and increases the *retention of administered amino acids and that a normal liver is necessary for these effects* (4) The action of growth hormone on glucose assimilation and on amino acid assimilation and retention occurs in the absence of the thyroid gland

General Discussion

JOHN C BECK (Royal Victoria Hospital) For my associates, Drs Venning J S L Browne Singer and Gareau I would like to present some data which is preliminary and highly confusing to us We feel somewhat as Dr Raben did this morning—we would like to let you in on the confusion Perhaps some of you can either substantiate or critically question the observations which I am about to show

For a number of years we have been interested in a bio assay of the sodium retaining factor and from some experiments which I am about to discuss we have obtained evidence that growth hormone or a preparation labeled as purified growth hormone leads to an increased production of the sodium retaining factor We believe that this measure of the sodium retaining factor is actually a measure of urinary aldosterone production

In Figure 17 are shown data from 4 normal males who received a growth hormone preparation supplied to us by the Horner Company The hormone was prepared by both the Wilhelmi procedure using ox pituitaries and the Raben Westermeyer procedure using pig pituitaries We have charted the time against the excretion of the sodium retaining factor which has been expressed in micrograms per hour equivalent to desoxycorticosterone The assays were made on the free fraction with one exception and this was made on the total fraction i e the free and conjugated steroids As you will note (Fig 17) in patient D K we found no appreciable excretion of the sodium retaining factor during the control period After the intravenous administration of 125 mg of the growth hormone preparation over approximately 30 minutes we found a significant increase in the excretion of the sodium retaining factor A similar result was obtained in the second patient W W we used the same preparation and the same general experimental procedure In the third patient we found a small amount of the sodium retaining factor in the control period and following the administration of the Raben Westermeyer growth preparation of pig pituitary we again observed a very definite and significant increase in the excretion of

E W FRANDSEN * (Medical College of Virginia) I believe the increased levels of 17 OH-corticosteroids Dr Moore has found after the administration of growth hormone can be satisfactorily explained by ACTH-contamination especially where the Raben preparation is concerned Using Dr Raben s procedure for precipitating GH from the ACTH-oxycellulose filtrate I have seen as much as 0 07 USP unit of ACTH per mg of GH Thus if 200 mg of such a GH preparation had been administered the patient would have received simultaneously an intravenous infusion of some 14 USP units of ACTH

Our Swedish GH preparation Somacton from which this ACTH contamination has been removed was administered daily to twelve patients with pulmonary tuberculosis without any significant increase in urinary excretion of 17 OH corticosteroids or 17 ketosteroids There was no alteration in the pattern of the excreted steroids which were determined after 2 7 14 and 44 days of treatment

The clinical results in these patients might be of interest in view of Dr Moore s negative results in surgical convalesence The daily dose was 10–15 mg which was equivalent to 500 units of a chosen unit the tibia unit The activity of the preparation was about equal to a preparation from Dr Li s laboratory which was used as a standard In addition 8–16 IU of insulin were administered twice daily The patients were treated from 5 to 22 weeks the average was 15 weeks All of them had advanced destructive pulmonary tuberculosis Four of the patients showed improvement before the treatment started The remaining eight patients had far advanced tuberculosis and were in a critical condition Weight loss was considerable and no improvement was evident in spite of prolonged treatment and intensive chemotherapy

Following the administration of GH no significant effect on the inflammatory state was apparent but there was a definite improvement in the general condition of all but one patient The appetite increased and the mental and physical vitality showed considerable improvement All but two patients showed a slow increase in body weight which increase ranged from 4 to 9 3 kg with an average of 6 6 kg at the end of the treatment No edema was observed and body weights did not show a rapid fall when the treatment was stopped A slight increase in fasting blood sugar (max 150 mg per cent) was noted but no glycosuria resulted Otherwise the GH had little effect on the various clinical and laboratory tests

A few similar cases have been treated with insulin alone and some with GH alone No effect comparable to what was seen with the combined treatment has been observed Further experiments are in progress and will be published by B Carstensen F Paulsen and I Roos from Sollidens Sanatorium Ostersund and from the Department of Pharmacology University of Lund Sweden

* Previously of the Nordic Hormone Laboratory Malmoe Sweden

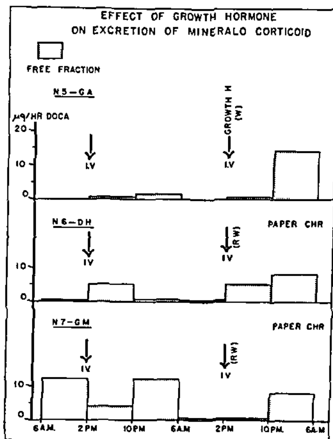


FIG 18

infusion over a 30 minute period there was definite increase in the sodium retaining factor The difference between these data and those of the previous group is that the induced effect seems to be delayed when the growth hormone is administered at 2 in the afternoon A similar result was obtained in the second experiment of this group (patient D H) Here we went further and separated the sodium retaining factor on two chromatographic systems namely the Zaffaroni and the Bush Again we feel that there was a definite response although it is not as convincing as found in the first patient of this second series Finally in the third patient G M there are findings which leave us totally confused Very high levels of sodium retaining factor were found in the control period and this we had not seen previously Following the administration of a Raben Westermeyer growth preparation then available to us we observed no response The reason for this one failure in the 7 patients of the series we are unable to say One problem of great trouble to us is that some of the growth hormone preparations have not been soluble at a physiological pH range and at best we are administering a suspension

E W FRANDSEN * (Medical College of Virginia) I believe the increased levels of 17 OH corticosteroids Dr Moore has found after the administration of growth hormone can be satisfactorily explained by ACTH contamination especially where the Raben preparation is concerned Using Dr Raben s procedure for precipitating GH from the ACTH oxycellulose filtrate I have seen as much as 0 07 USP unit of ACTH per mg of GH Thus if 200 mg of such a GH preparation had been administered the patient would have received simultaneously an intravenous infusion of some 14 USP units of ACTH

Our Swedish GH preparation Somacton from which this ACTH contamination has been removed was administered daily to twelve patients with pulmonary tuberculosis without any significant increase in urinary excretion of 17 OH corticosteroids or 17 ketosteroids There was no alteration in the pattern of the excreted steroids which were determined after 2 7 14 and 44 days of treatment

The clinical results in these patients might be of interest in view of Dr Moore s negative results in surgical convalescence The daily dose was 10–15 mg which was equivalent to 500 units of a chosen unit the tibia unit The activity of the preparation was about equal to a preparation from Dr Li s laboratory which was used as a standard In addition 8–16 IU of insulin were administered twice daily The patients were treated from 5 to 22 weeks the average was 15 weeks All of them had advanced destructive pulmonary tuberculosis Four of the patients showed improvement before the treatment started The remaining eight patients had far advanced tuberculosis and were in a critical condition Weight loss was considerable and no improvement was evident in spite of prolonged treatment and intensive chemotherapy

Following the administration of GH no significant effect on the inflammatory state was apparent but there was a definite improvement in the general condition of all but one patient The appetite increased and the mental and physical vitality showed considerable improvement All but two patients showed a slow increase in body weight which increase ranged from 4 to 9 3 kg with an average of 6 6 kg at the end of the treatment No edema was observed and body weights did not show a rapid fall when the treatment was stopped A slight increase in fasting blood sugar (max 150 mg per cent) was noted but no glycosuria resulted Otherwise the GH had little effect on the various clinical and laboratory tests

A few similar cases have been treated with insulin alone and some with GH alone No effect comparable to what was seen with the combined treatment has been observed Further experiments are in progress and will be published by B Carstensen F Paulsen and I Roos from Sollidens Sanatorium Ostersund and from the Department of Pharmacology University of Lund Sweden

* Previously of the Nordic Hormone Laboratory Malmoe Sweden

Drs S Nordstrom and F Paulsen, at the General Hospital Malmoe Sweden have used the same GH preparation Somacton in a series of patients who developed psychotic reactions during corticotropin therapy Their observations suggest that this GH preparation often reverses or obliterates the psychotic symptoms, at times rapidly, without interfering with the beneficial or eosinopenic effect of the corticotropin

30

Closing Remarks

C N H Long
Yale University School of Medicine New Haven

It must be obvious to all who have heard the papers given in this room during the last three days that any attempt to draw final conclusions concerning the chemistry or the physiological and metabolic effects of the growth hormone would not only be untimely but a task beyond the power of any one individual to accomplish The failure of any such attempt is obviously not due to lack of experimental data indeed one of the most impressive aspects of this meeting is the realization that such a large number of individuals on both sides of the Atlantic are engaged in such a variety of studies on this hormone Rather it is to be attributed to certain unique peculiarities of the organ that produces this agent

While the terms master gland and conductor of the glandular orchestra are dearly loved by popularizers of science they do contain certain elements of truth There has not been much discussion at this meeting of what I am sure we would all agree is an outstanding feature of anterior pituitary function I refer of course to the role of this gland in the regulation of other endocrines by means of tropic hormones while still retaining the capacity to secrete agents that exert their effects directly on tissues other than those of an endocrine character This together with the added fact that the secretions of target endocrine organs such as the adrenal cortex can in turn influence the rate of secretion of the tropic hormone confers on the anterior pituitary an extraordinary degree of control over a variety of bodily functions It is through this that it exerts a regulatory influence on both protein anabolism and catabolism on blood glucose levels and a variety of other metabolic phenomena Such changes can be brought about in theory at least by a reduction in the secretion of one agent leaving the secretion of the other at a normal rate or by an actual augmenta

tion of secretion of one agent alone As an example of the former, I can cite certain evidence mentioned here on several occasions that adrenalecto mized animals behave in some ways like normal or hypophysectomized animals given an excess of growth hormone

This takes us at once to a central problem *in anterior lobe physiology* which also has not been discussed at this conference This is the question as to what regulates the secretion of growth hormone Is the growth of a rat or man from birth to the attainment of adulthood accompanied first by an increased proportion of growth hormone in the secretion of the anterior lobe followed by a decline in later years or is the whole phenom enon of the endocrine control of growth merely a progressive change in the sensitivity of the tissues to this agent? If it is the former then what is it in the growth process that stimulates the pituitary to supply the extra quantities of growth hormone required and by the same token what changes occur in later life that dampen this secretion?

On the other hand if the blood level of growth hormone is constant *throughout life then what is the nature of the change in tissue sensitivity* to its presence?

While such questions cannot be completely answered at present they suggest that there may exist mechanisms within the central nervous system or the gland itself which are influenced by the composition of the blood passing through them a blood which may change from hour to hour day to day or month to month in its content of hormones or products of metabolism

A first approach to this intricate phenomenon lies in the development of methods for the detection of growth hormone in blood a matter briefly mentioned by Dr Segaloff but otherwise passed over Surely the develop ment of such a method would be of the greatest value in the resolution of many problems that have offered contradictions to us during the last three days There has been much discussion for example on the failure of ox growth hormone to cause growth and metabolic changes in certain species What do we know of the lifespan of this agent in the blood stream of resistant species as compared to those that are sensitive to its effects Dr Li and his colleagues have estimated the half life of the hormone in the blood of the rat to be several hours What is it in man?

Years of work have gone into the problems of assay of growth hormone and these have been carefully and thoughtfully reviewed by Drs Geschwind and Russell Nevertheless I am left with the impression that there is no general satisfaction with the specificity or accuracy of any method used at present This view is strengthened when one considers what has been the source of the major contradictions at this meeting I refer of course to the debated question of the absolute purity of any growth hormone *preparation* yet prepared and to the ancillary problem as to how many hormones con tribute to the effects observed Until this is settled it would seem difficult

to devise an assay method which would specifically indicate with sufficient sensitivity and accuracy the presence of growth hormone in the body fluids

The question of the need of other hormones to obtain growth effects has been debated in terms of permissiveness of synergism and of absolute requirement Indeed it has been pointed out that under certain conditions all hormones are growth hormones This is true but in many instances only when the hormone is given to an animal suffering from a deficiency of that agent Excess of cortisone or thyroxin is not growth promoting in intact animals while growth hormone has this unique characteristic

A more extreme view is that growth hormone does not act directly on the tissues but is itself a tropic agent Dr Selye has suggested on other occasions if not at this meeting that somatotropin which I believe he identifies as growth hormone causes the secretion of mineralo-corticoids We have heard this afternoon a short statement from Dr Beck and the Montreal laboratory with data supporting this viewpoint Dr Best on the other hand has suggested that insulin is the real excitor of protein anabolism and growth and that the so-called growth agent is isletotropic

This discussion so far as it was carried left me with the tentative conclusion that we should not throw away the baby before we are sure of the composition of the bath water It would seem self evident that nutritional vitamin mineral and hormonal deficiencies do not furnish the best internal environment for growth and reproduction and conversely that these processes may well require extra quantities not only of the right nutriments but of the other hormones needed for the conversion of these nutrients into the energy required by these processes

I could go on a long time reviewing my impressions and conclusions of the papers you have heard but there seems little point in doing so Furthermore I am no more capable than the rest in formulating answers without additional experimental data and it is overwhelmingly apparent that this is what is needed The purpose of the conference was to enable all of us to assess the present evidence on each of the matters under discussion and it would be a fruitless conference if it had left us all agreed that all was known about this hormone

Opinions will differ on interpretation as well as on the strength and validity of the evidence laid before us The disclosure of such differences of opinion and the stimulus to return home and do more work was the purpose of our meeting In both these regards it has been outstandingly successful

Let me close by taking a leaf from Dr Astwood's book and express a little of my own philosophy about endocrinology The papers given today have emphasized what should never be forgotten which is that the hormones do not initiate cellular reactions or metabolic transformations These are intrinsic properties of the cells alone and the hormones merely alter the rate at which certain changes occur Indeed in some instances the maximal

change in activity produced by a hormone may be only a small fraction of that going on in the unstimulated cell Nevertheless, these slight displacements if long continued can lead to profound overall changes

Dr Cori reviewed the central questions as to the site of action of hormones on cells, whether these were effects on permeability or on enzyme systems *This is an exciting field but I would like to remind you that the* papers we heard on the mode of action of insulin could not have been given without the prior knowledge of the mechanism of carbohydrate degradation and synthesis in cells That is why I believe we will not know all we wish to know about the effects of growth hormone on protein metabolism until we understand a great deal more of the intermediary metabolism of protein in the cells As this knowledge is acquired I am confident that much of our confusion about the action of growth hormone will disappear

All this emphasizes that the future of endocrinology is inextricably linked with biochemistry and that we are in a transitional stage between the descriptive type of endocrinology and the time when we can speak of hormones in terms of their effect on metabolism This has already occurred in the case of vitamins and I am sure it will also be true for the hormones So let us not be too discouraged by the present contradictions and inconsistencies

Finally, I would like to act as your spokesman in expressing to Dr Buerki and the Trustees of the Ford Hospital and Edsel Ford Institute as well as to the Local Committee and staff of the Hospital our sincere appreciation of the opportunity they have given us at this conference to meet and exchange ideas on this topic and to thank them for all they have done to make our meeting so enjoyable The Hospital and Institute are making a very real contribution to medical science by holding meetings such as this and I think that we as a representative group of medical scientists should place this in the records of the meeting